PARASITOLOGY

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PARASITOLOGY

(Founded by G. H. F. Nuttall, F.R.S.)

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VOLUME XXVIII JANUARY, 1936 No. 1

THE PROSTIGMATIC MITES OF SOUTH AFRICAN LIZARDS

BY R. F. LAWRENCE, PH.D.

Assistant in Charge of Arachnida, South African Museum, Cape Town

(With 23 Figures in the Text)

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I. INTRODUCTION

IN a previous paper (1935) an account was given of the prostigmatic mites parasitic on South African lizards, belonging to the two endemic families, the Zonuridae and Gerrhosauridae. The present paper deals with the fauna of the two remaining families of lizards, the Geckonidae and Agamidae, parasitised exclusively by prostigmatic mites. Trägardh (1905) and Hirst (1926) have made the most considerable contributions to the knowledge of the parasites of Geckonid and Agamid lizards; the latter placed the group on a sound systematic basis, the former, in addition to his descriptions of new forms, contributed valuable notes on the development and morphology of the species studied. With regard to mite parasites, however, up to the present, no systematic investigation has been attempted of all the species belonging to any one family of lizards from a given zoo-geographical region. In the present paper this has been attempted; the great majority of Geckonid and all the species of Agamid lizards from the South African subregion, either as living or spirit specimens, have been examined; from most of them mite parasites

have been obtained. Both families of lizards are, genealogically speaking, derived from a long-established Saurian stock; both are, in South Africa, extremely well demarcated, homogeneous families, with their main structural characteristics varying within narrow limits. In neither of them does loss or degeneration of the limbs occur. Each family is parasitised by a single genus of prostigmatic mites, the Geckonidae by *Geckobia*, the Agamidae by *Pterygosoma*; while *Geckobia* is found on the Geckonidae and on no other family, *Pterygosoma* is also a parasite of Gerrhosaurid lizards on which three species have been found. In a previous paper by the writer (1935, p. 3) *Pimeliaphilus* was cited as occurring on Geckonidae. Trägardh, who erected the genus (1905, p. 31), actually found his species *P. podapolipophagus* living under the elytra of a beetle, *Pimelia*, but the other two species of the genus, *P. insignis* Berl. and *P. tenuipes* Hirst, both occur on Geckonid lizards. Neither of these two species have been found in South Africa and it is extremely unlikely that they occur in this region.

II. MITES PARASITIC ON SOUTH AFRICAN GECKONIDAE

Genus *Geckobia* Mégnin

(Figs. 1 and 2)

1878. Mégnin, *Bull. Soc. ent. Fr.* Sér. 5, **8**.

Adult. Dorsal surface of body almost entirely covered with hairs which are usually cylindrical, rarely flattened and paddle-shaped as in *Pterygosoma* and *Zonurobia*, and which may be plumose or simple; dorsal scute present, eyes fairly distinct. Ventral surface usually with more or less cylindrical hairs, rarely with flattened scale-like hairs. Mouth-parts nearly always short and stout, almost wholly exposed on the anterior dorsal surface of the body, not covered basally by integument as in *Zonurobia*; segment II of the palpi (the "first free segment" of Hirst) nearly always armed dorsally with a plumose incrassate hair; hypostome with its sides curved upwards to form a shallow conduit, tending towards the condition found in *Ixodiderma*. Anal field generally situated on the dorsal surface, the arrangement and structure of the hairs associated with it not differing among the species, forming a pattern more or less stereotyped throughout the genus. Legs differing from all other Pterygosomid genera in having their coxae armed with large thickened hairs (Fig. 1), the "coxal spurs" of Hirst; these spurs completely or incompletely plumose, stout and short or long and slender, in the latter case differing very little from the incrassate hairs of the distal segments; five of these spurs occur on the posterior common coxa, three corresponding to leg IV and two to leg III; anterior common coxa with two spurs corresponding to leg II and two much finer hairs corresponding to leg I which may be simple and bristle-like, or plumose; in legs I–III these hairs and spurs are similarly situated, one being at the apex of the coxa near the insertion of the trochanter, the other at the base; in leg IV the three spurs form a row along the posterior margin of the common coxa. Legs differing further from other genera in having

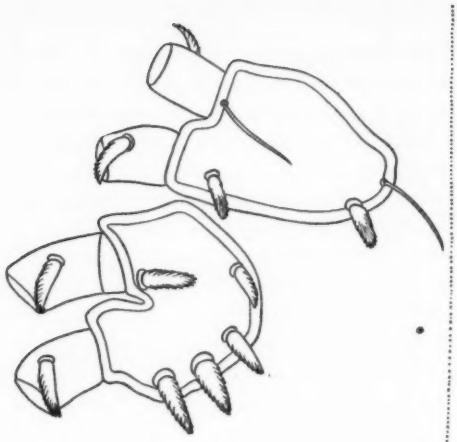


Fig. 1. *Geckobia homopholis*. Adult. Coxae of legs. The dotted line indicates the longitudinal axis of the body.

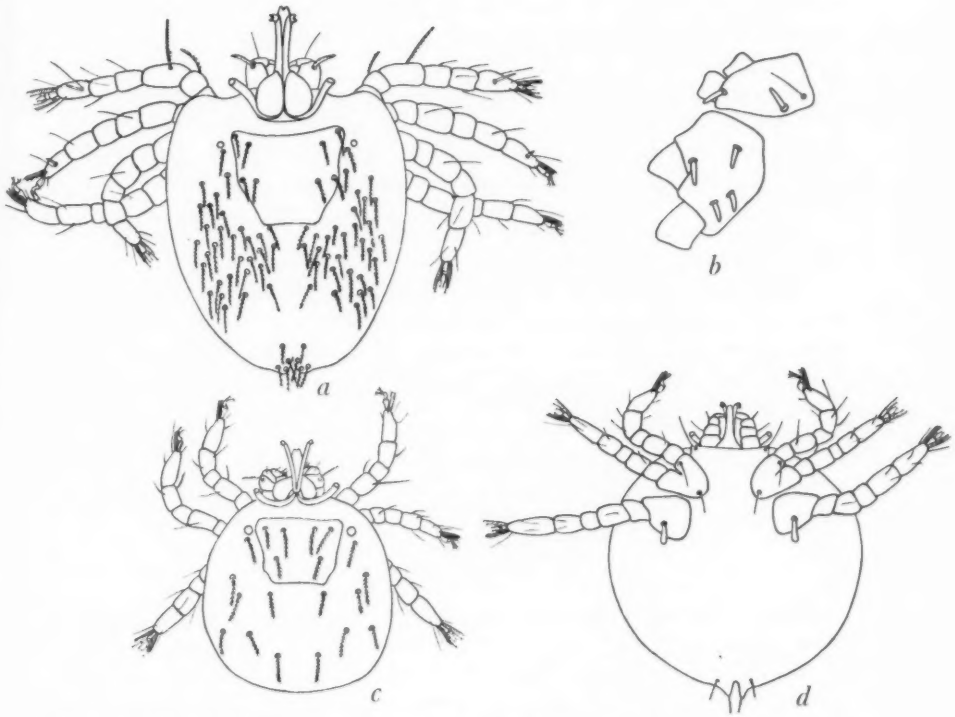


Fig. 2. *Geckobia phyllodactyli*. *a*, dorsal view of nymph; *b*, coxae of nymph; *c*, dorsal view of larva; *d*, ventral view of larva (a different specimen from that of Fig. 2*c*).

the first two segments following the common coxa, *i.e.* trochanter and femur, provided anteriorly with a large incrassate cylindrical hair which is generally plumose. In the normal condition these hairs are present on these two segments in legs I, III and IV; in II a hair is present only on the trochanter. Exceptions from the normal are (i) the presence of this hair on the femur of leg II, (ii) the absence of a hair on the femur of leg III, (iii) the presence of a third hair on the segment following the femur in leg IV; in certain cases a fourth hair may be present on the penultimate segment of leg IV. All these exceptions are constant for a species and provide good diagnostic characters. The claw in *Geckobia* is in general stouter, shorter, and more compact than in *Zonurobia* and *Pterygosoma*, though this structure is essentially the same throughout the family Pterygosomidae. The body size in *Geckobia* is much smaller than in other genera of Pterygosomidae; the breadth is nearly always considerably greater than the length.

Nymph as in Fig. 2*a*, the body apparently longer than broad; scute and eyes present; hairs on dorsal and ventral surface plumose; mouth-parts and spination of legs as in adult; coxae (Fig. 2*b*) differing from the adult in having one coxal spur less on the posterior common coxa and one spine less on the anterior common coxa, thus four spurs on the former, and two spurs and a spine on the latter.

Larva as in Fig. 2*c*, seen from above, about as broad as long; mouth-parts as in adult; dorsal hairs plumose; anal field present though undeveloped, a median opening present; coxae of legs as in Fig. 2*d*; posterior coxa with only one large spur, anterior common coxa with two spines.

Geckobia australis Hirst

1926. Hirst, S., *J. Linn. Soc.* (*Zool.*), **36**, 176, Fig. 3.

This species seems to be found only on *Lygodactylus capensis capensis* and is the most common scale mite in the eastern half of South Africa. It has been taken on the above-named Gecko from the following localities:

Transvaal: Hectorspruit, near Komatipoort; Mariepskop; Palmyraville, Zoutpansberg; Roodepoort, Pretoria District; Komatipoort; Rustenburg; Vygeboompoort.

Rhodesia: Salisbury and one unknown locality.

Hirst's types were from Beira, Portuguese East Africa.

Geckobia ovambica n.sp.

(Fig. 3)

Types. Two specimens from *Rhoptropus barnardi*, Kunene River, South-West Africa.

Body not much broader than long. Dorsal surface as in Fig. 3*a*, the hairs all of the same type, fairly long and slender, those in the middle of the anterior part of the body about half as long as the peripheral hairs and slightly plumose (Fig. 3*b*), the peripheral hairs smooth (Fig. 3*c*). Ventral surface

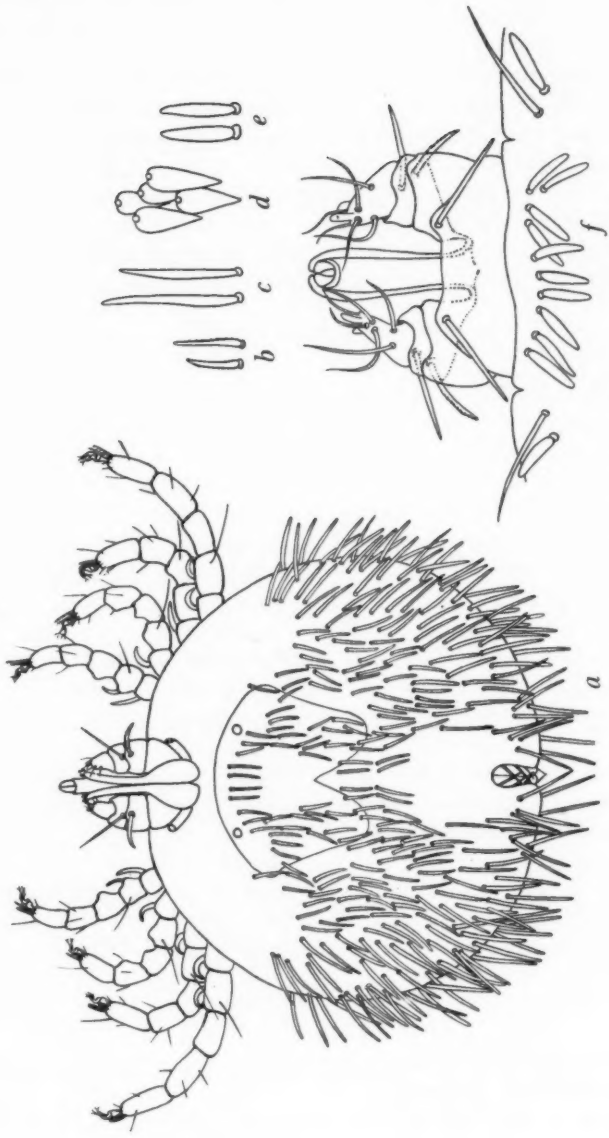


Fig. 3. *Geckobia ovambica*. Adult. *a*, dorsal view; *b* and *c*, hairs of the dorsum; *d*, ventral hairs; *e*, coxal spurs; *f*, mouth-parts, ventral view.

entirely covered in the middle with pointed, flattened, scale-like hairs (Fig. 3*d*); peripheral hairs posteriorly and at the sides much more cylindrical, almost similar in shape to the dorsal peripheral hairs; the most anterior row of hairs as in Fig. 3*f*.

Mouth-parts normal, the dorsal hair on the second segment of the palp not much stouter than that on the third segment and very slightly plumose. The structures of the mouth-parts seen from below armed as in Fig. 3*f*, the apical segment of the palps with a long cylindrical blunt-tipped claw.

Legs equal-sized, differing from the normal in the incrassate hair on segment II of leg II being absent, these hairs very slightly or not at all plumose. Coxal spurs long and slender (Fig. 3*e*), not plumose but striated longitudinally.

Dimensions. Length of body 0·42 mm., breadth 0·47 mm.

Geckobia tasmani n.sp.

(Fig. 4)

Types. Eight specimens from *Hemidactylus tasmani*, Chishawasha, near Salisbury, Rhodesia.

Body definitely broader than long. Dorsal surface of body as in Fig. 4 *a*, the dorsal hairs of three sizes as in Figs. 4 *b*, *c*, *d*; the spine-like hairs at and near the periphery smooth, not plumose (Fig. 4 *d*). Ventral surface with hairs similar to those of the dorsal surface, the great majority being long, cylindrical and spine-like; anteriorly a few smaller hairs resembling those of Fig. 4 *b*, a narrow strip just below the mouth-parts and coxae free of hairs.

Mouth-parts. Seen from above, the second segment of the palps differing from all other South African species of *Geckobia* in having a long, slender, and simple bristle, instead of the usual incrassate and plumose hair; this bristle as long as, or a little longer than, the similarly formed bristle on the segment distal to it.

Legs equal-sized, differing from all other South African species in having the incrassate plumose hair of the first and second segments replaced by a simple bristle. Coxal spurs as in Fig. 4 *e*.

Dimensions. Length of body 0·54 mm., breadth 0·54 mm. Although the measurements of the specimen drawn are as given above, distortion in the process of mounting has increased the length out of proportion to the breadth; all other specimens are considerably broader than long.

Geckobia oedurae n.sp.

(Fig. 5)

Types. Twenty-five specimens from *Oedura transvaalica platyceps*, Empandeni, near Salisbury, Rhodesia.

Body usually a little broader than long. Dorsal surface of body as in Fig. 5 *a*, the hairs all of a more or less uniform size (the anterior ones (Fig. 5 *b*) a little shorter), cylindrical, slender, and smooth (the anterior ones more or less plumose). Peripheral hairs as in Fig. 5 *c*. Dorsal scute very indistinctly

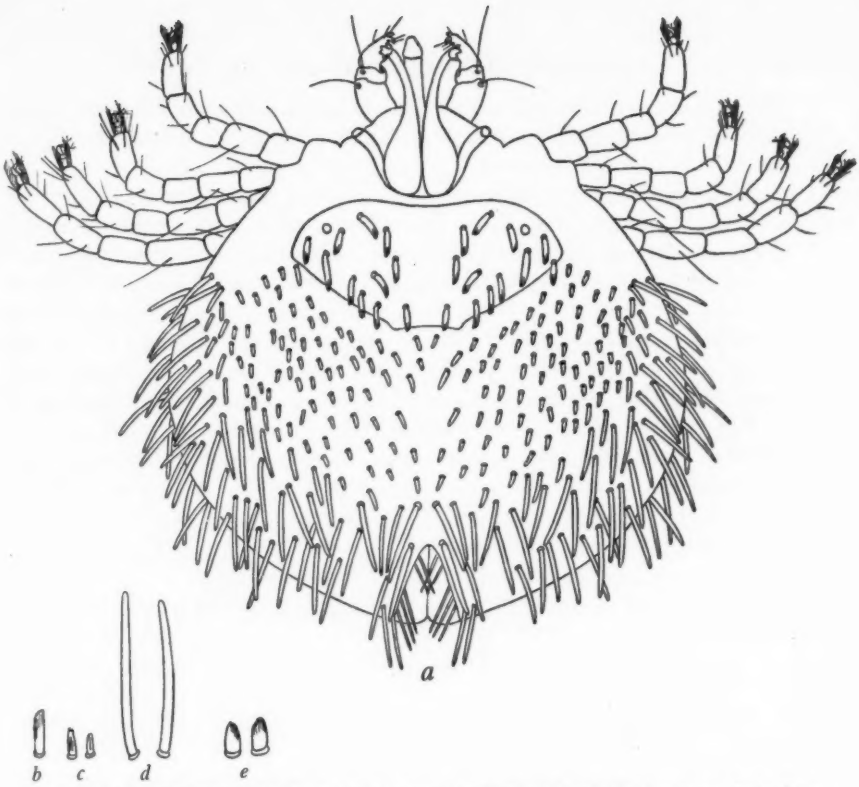


Fig. 4. *Geckobia tasmani*. Adult. *a*, dorsal view; *b*, anterior hair of dorsal scute; *c*, hairs from middle of the dorsum; *d*, peripheral hairs; *e*, coxal spurs.

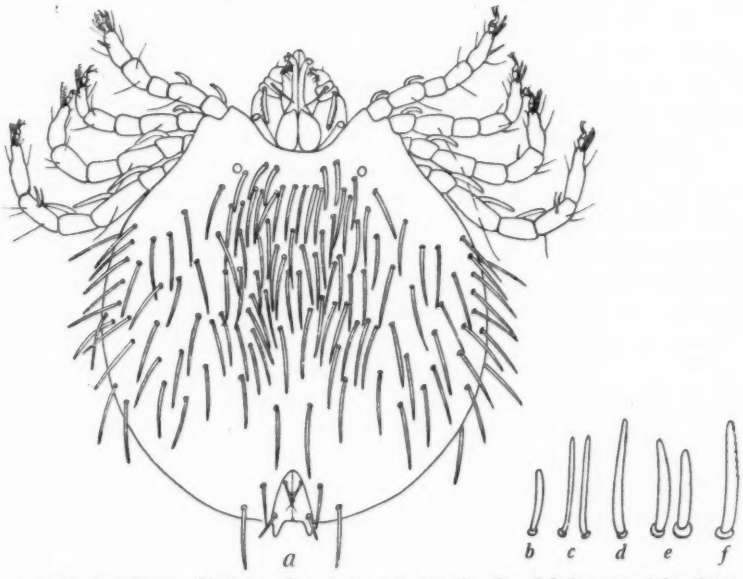


Fig. 5. *Geckobia oedurae*. Adult. *a*, dorsal view; *b*, anterior dorsal hair; *c*, peripheral hairs; *d*, ventral hair; *e*, coxal spurs; *f*, incrassate hair on segment I of leg IV.

defined. Ventral surface much more densely covered with hairs than the dorsal surface, these smooth (Fig. 5 *d*), of the same general type as the dorsal peripheral hairs but more lanceolate.

Mouth-parts normal, deeply sunk in a concavity of the anterior margin of the body, the dorsal hair on the second segment plumose, two-thirds the length of that on the segment distal to it, which is simple and setiform.

Legs equal-sized, differing from all other South African species of *Geckobia* in having (i) segment III of leg IV with a plumose incrassate hair, (ii) the incrassate hair on segment II of leg III absent, (iii) segment IV of legs III and IV with a *pair* of slender incrassate hairs anteriorly, these larger in IV than in III. There are thus incrassate hairs on the four proximal segments of leg IV instead of the usual two proximal segments; these incrassate hairs are, moreover, unusually large and conspicuous, being in some cases longer than the segments on which they are situated. Coxal spurs long and slender (Fig. 5 *e*), hardly differing in size or appearance from the incrassate hairs of the remaining segments of the legs (cf. Figs. 5 *e* and 5 *f*).

Dimensions. Length of body 0·4 mm., breadth 0·44 mm.

Other records. From *Oedura transvaalica platyceps*, Driefontein, Southern Rhodesia.

Geckobia natalensis n.sp.

(Fig. 6)

Types. Sixteen specimens from *Lygodactylus capensis capensis*, Umhlatu, Natal.

Body a little broader than long. Dorsal surface as in Fig. 6 *a*; the dorsal hairs of uniform size, stout, moderately pointed, and smooth (Fig. 6 *c*), except a few near the anterior margin which are plumose (Fig. 6 *b*). The anal field in Fig. 6 *a*, more or less conjectural, as this portion of the body cannot be clearly seen in the specimens mounted. Ventral surface with hairs similar to those of the dorsal surface or a little stouter (Fig. 6 *d*), distributed evenly over almost the entire under-surface.

Mouth-parts normal.

Legs equal-sized and normally armed; the plumose incrassate hair on segment I of leg IV the largest of these hairs, that on segment II of leg III the smallest. Coxal spurs as in Fig. 6 *e*, long, slender, and only plumose at the apices, if at all.

Dimensions. Length of body 0·48 mm., breadth 0·53 mm.

Other records. From *Lygodactylus capensis capensis*, Mozambique.

Geckobia hewitti n.sp.

(Fig. 7)

Types. Thirty-seven specimens from *Pachydactylus maculatus maculatus*, Resolution Halt, Grahamstown.

Body broader than long. Dorsal surface as in Fig. 7 *a*, all the hairs plumose, the peripheral ones (Fig. 7 *d*) more so than in other species. Anterior hairs

of the scute (Fig. 7 *b*) usually a little shorter but considerably broader than those of the middle of the body (Fig. 7 *c*). Ventral surface with long, slender, very slightly flattened, lanceolate hairs (Fig. 7 *e*), these smooth and a little longer than the peripheral hairs of the dorsal surface, those of the anterior part of the body shorter, more cylindrical, and slightly plumose. Anterior margin of the body in the middle just below the mouth-parts as in Fig. 7 *g*,

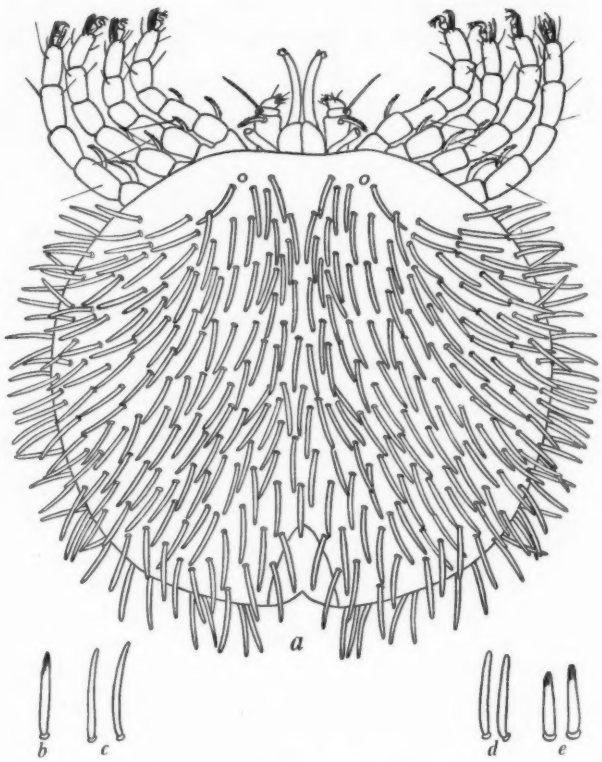


Fig. 6. *Geckobia natalensis*. Adult. *a*, dorsal view; *b*, anterior dorsal hair; *c*, peripheral hairs; *d*, ventral hairs; *e*, coxal spurs.

the ventral chitinous plate of the mouth-parts with two long slender spines situated on conspicuous projections.

Mouth-parts normal, seen from above the second segment of the palps with an unusually stout plumose hair, the palps themselves short and incrassate.

Legs. The posterior legs a little longer but hardly stouter than the anterior legs. They differ from the normal in having (i) segment II of *all* legs provided

with a plumose incrassate hair, (ii) segment III of legs I and IV with an anterior hair, that of IV plumose, of I simple. Coxal spurs as in Fig. 7 *f*.

Dimensions. Length of body 0·42 mm., breadth 0·5 mm.

Other records. From the same host, locality unknown.

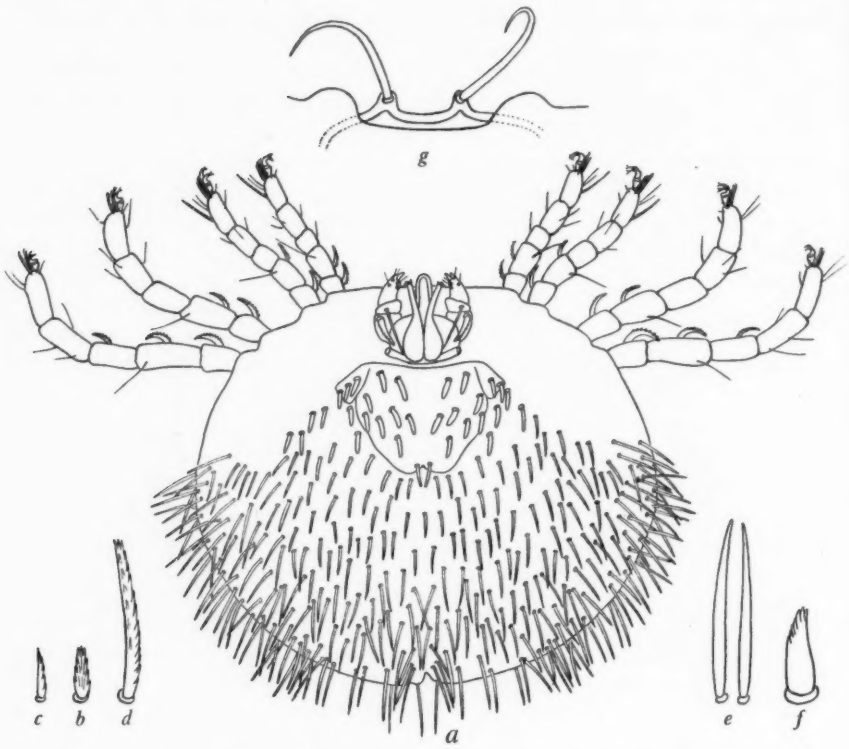


Fig. 7. *Geckobia hewitti.* Adult. *a*, dorsal view; *b*, anterior dorsal hair; *c*, hair from the middle of the dorsum; *d*, peripheral hair; *e*, ventral hairs; *f*, coxal spur; *g*, ventral plate of mouth-parts, ventral view.

Geckobia namaquensis n.sp.

(Fig. 8)

Types. Twenty-five specimens from *Pachydactylus namaquensis*, Garies, Namaqualand.

Body broader than long, proportionally broader in most specimens than is represented in Fig. 8 *a*. Dorsal surface as in Fig. 8 *a*, the hairs of the dorsal scute (Fig. 8 *c*) broader but not much longer than those in the middle of the body (Fig. 8 *b*), both these types of hair plumose; the peripheral hairs (Fig. 8 *d*) cylindrical, bluntly pointed, and not or hardly plumose. Ventral surface with

slightly flattened, smooth, lanceolate hairs (Fig. 8 *e*), those more anteriorly situated shorter, cylindrical, and plumose.

Mouth-parts sunk in a deep concavity of the anterior margin of the body.

Legs normal, the incrassate hairs on the first segments plumose along their entire length. Coxal spurs long and slender, plumose in their distal halves (Fig. 8 *f*).

Dimensions. Length of body 0·4 mm., breadth 0·4 mm.

Other records. From the same host, Steinkopf, Namaqualand; from *Pachydactylus capensis gariesensis*, Garies, Namaqualand.

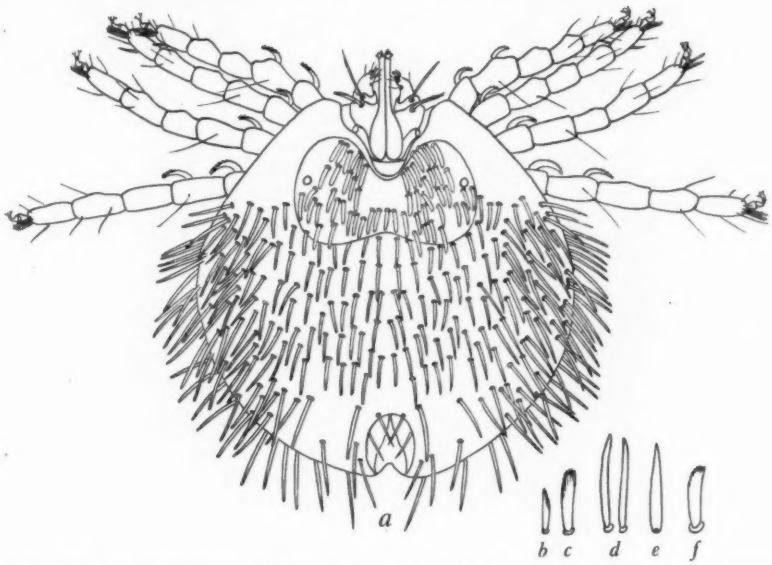


Fig. 8. *Geckobia namaquensis.* Adult. *a*, dorsal view; *b*, hair from middle of the dorsum; *c*, anterior dorsal hair; *d*, peripheral hairs; *e*, ventral hair; *f*, coxal spur.

Geckobia rhoptropi n.sp.

(Fig. 9)

Types. Six specimens from *Rhoptropus ocellatus*, Leliefontein, Namaqualand.

Body about as broad as long, or not much broader than long. Dorsal surface with hairs as in Fig. 9 *c*, uniform in size and shape, smooth and more or less cylindrical except a few anteriorly on the scute which are truncated apically and slightly plumose (Fig. 9 *b*); anal field seen from above as in Fig. 9 *e*. Ventral surface with hairs similar to those of the dorsal surface.

Mouth-parts normal.

Legs more or less equal-sized, III and IV a very little longer than I and II, but not incrassate. Plumose incrassate hairs of segments I and II much

weaker than usual. Coxal spurs as in Fig. 9 *d*, long and slender, more or less similar in length and thickness to the anterior hairs of the dorsal surface.

Dimensions. Length of body 0·48 mm., breadth 0·5 mm.

Other records. From *Lygodactylus bradfieldi*, Okahandja, South-West Africa.

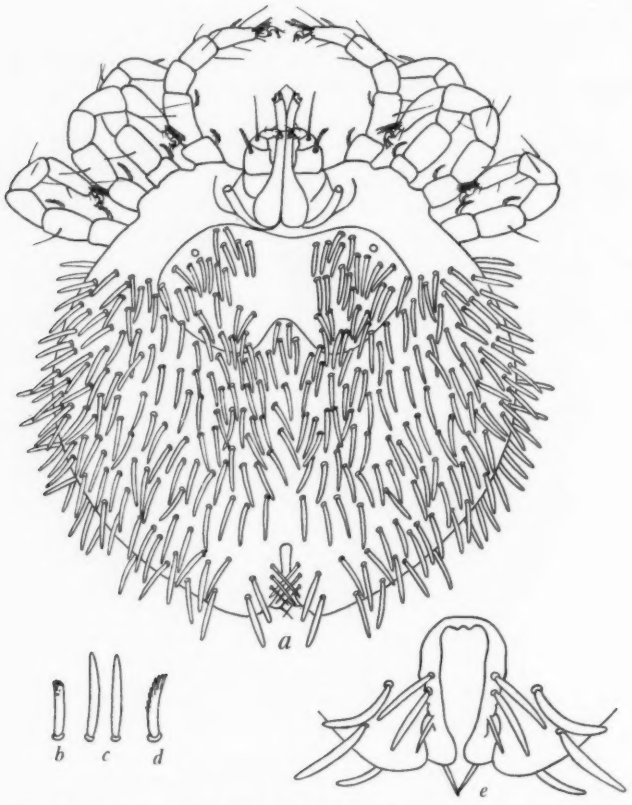


Fig. 9. *Geckobia rhoptropi*. Adult. *a*, dorsal view; *b*, anterior dorsal hair; *c*, peripheral hairs; *d*, coxal spur; *e*, anal field, dorsal view.

Geckobia pachydactyli n.sp.

(Fig. 10)

Types. Thirty specimens from *Pachydactylus bibronii bibronii*, Bitterfontein, Namaqualand.

Body considerably broader than long. Dorsal surface of body as in Fig. 10 *a*, the hairs on the scute differing in size, the two median anterior hairs (Fig. 10 *b*), broader than the others; two other hairs just laterally to these considerably longer than any of the remaining hairs of the scute; hairs

in the middle of the body small, more or less plumose, those of the periphery, Fig. 10 *c*, long, cylindrical, and slightly plumose, distinctly longer than the hairs on the scute. Ventral surface, except the coxae, almost entirely covered with hairs, the great majority of these slightly expanded and lanceolate, Fig. 10 *d*, their surfaces longitudinally striated and slightly plumose. Anterior hairs of the ventral surface not lanceolate but resembling the hairs of the dorsal scute in size and shape.

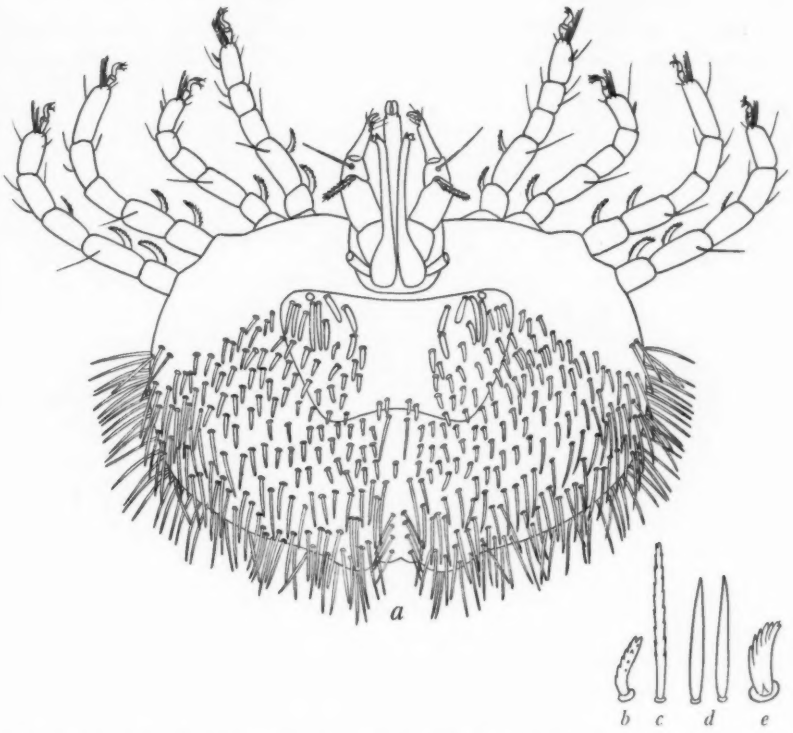


Fig. 10. *Geckobia pachydactyli*. Adult. *a*, dorsal view; *b*, anterior median hair of dorsum; *c*, peripheral hair; *d*, ventral hairs; *e*, coxal spur.

Mouth-parts differing from all other South African species in having the chelicerae, palps, and hypostome exceptionally long and slender; dorsal hair on the second segment of palp strongly plumose, about three-fourths the length of the hair on the segment distal to it, which is simple and setiform.

Legs equal-sized, the spination differing from the normal in having a long bristle-like hair on the anterior margin of segment III of leg I, also a very slightly plumose hair on segment III of leg IV. Coxal spurs stout, subtriangular, and apically plumose (Fig. 10 *e*).

Dimensions. Length of body 0·37 mm., breadth 0·55 mm.

Other records. From the same host, Aus, South-West Africa; Oup River, South-West Africa; Leliefontein, Namaqualand.

Geckobia hemidactyli

(Fig. 11)

Types. Sixty-five specimens from *Hemidactylus tasmani*, Driefontein, Southern Rhodesia.

Body much broader than long. Dorsal surface as in Fig. 11 *a*, the hairs of three types; anterior hairs on the dorsal scute (Fig. 11 *c*) broader and a

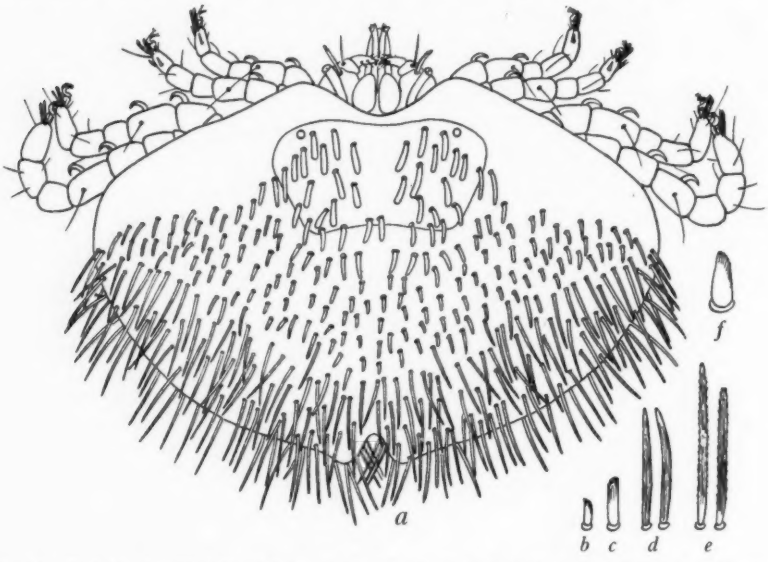


Fig. 11. *Geckobia hemidactyli*. Adult. *a*, dorsal view; *b*, hair from middle of the dorsum; *c*, anterior dorsal hair; *d*, ventral hairs; *e*, peripheral hairs; *f*, coxal spur.

little longer than those of the middle of the body, the latter longish triangular and slightly plumose (Fig. 11 *b*), peripheral hairs very long and distinctly plumose (Fig. 11 *e*). Ventral surface in the middle with very slighly flattened hairs (Fig. 11 *d*), not plumose but striated, a little shorter than the dorsal peripheral hairs, those of the anterior part of the ventral surface much shorter, cylindrical, and more or less plumose.

Mouth-parts normal.

Legs. The posterior pair distinctly longer and stouter than the anterior pair, spination normal, the incrassate hairs plumose along their whole length. Coxal spurs stout, plumose along their distal two-thirds (Fig. 11 *f*).

Dimensions. Length of body 0·4 mm., breadth 0·61 mm.

Other records. From *Hemidactylus tasmani*, Kutama, Southern Rhodesia; *H. mabouia*, Lourenco Marques; *H. mabouia*, Zambi, Belgian Congo.

Geckobia homopholis n.sp.

(Figs. 1 and 12)

Types. Twenty specimens from *Homopholis wahlbergii*, Bulawayo, Rhodesia.

Body considerably broader than long. Dorsal surface as in Fig. 12 *a*; hairs of three types, small, medium and long; hairs of the scute mostly of medium size, broader than the others and slightly plumose (Fig. 12 *c*), the small hairs of the middle of the dorsal surface subtriangular and very slightly plumose (Fig. 12 *b*); the peripheral hairs long, cylindrical, and smooth (Fig. 12 *d*). Ventral surface covered, except quite anteriorly, with hairs similar to those of the dorsal periphery, except that they are slightly more flattened basally, and more pointed apically (Fig. 12 *e*); anteriorly the ventral surface is provided with hairs resembling the small hairs of the dorsum but a little larger than these.

Mouth-parts normal, the hair on the second segment plumose, about half as long as that on the third segment.

Legs normal, the posterior pair a little longer, though not more incrassate, than the anterior pair; segment III of legs I and IV with a weak bristle anteriorly. Coxal spurs stout and comparatively short (Figs. 1 and 12 *f*).

Dimensions. Length of body 0·43 mm., breadth 0·62 mm.

Other records. From *Homopholis wahlbergii*, Salisbury; Geelhout Kop, Waterberg District, Transvaal; Waterval Boven, Eastern Transvaal.

Geckobia phyllodactyli n.sp.

(Figs. 2 and 13)

Types. Eight specimens from *Phyllodactylus porphyreus*, Hermanus, Cape Province.

Body broader than long, subquadrate. Dorsal surface as in Fig. 13 *a*; the anterior hairs of the scute a little stouter than those of the middle of the body, but hardly distinguishable from them (Fig. 13 *b*); both types weakly plumose; peripheral hairs long, cylindrical, and smooth, except at their apices where they are a little roughened (Fig. 13 *c*). Ventral surface covered with close-set, imbricating, distinctly flattened, lanceolate hairs (Fig. 13 *d*), not plumose but slightly striated longitudinally; those near the anterior margin of the body less flattened but nevertheless distinctly lanceolate and not resembling the anterior hairs of the dorsal surface.

Mouth-parts normal, the hair on the second segment of the palps more incrassate than usual.

Legs. Anterior common coxa more widely separated from the posterior common coxa than usual, the posterior legs distinctly longer and stouter than the anterior ones; spination differing from the normal in having

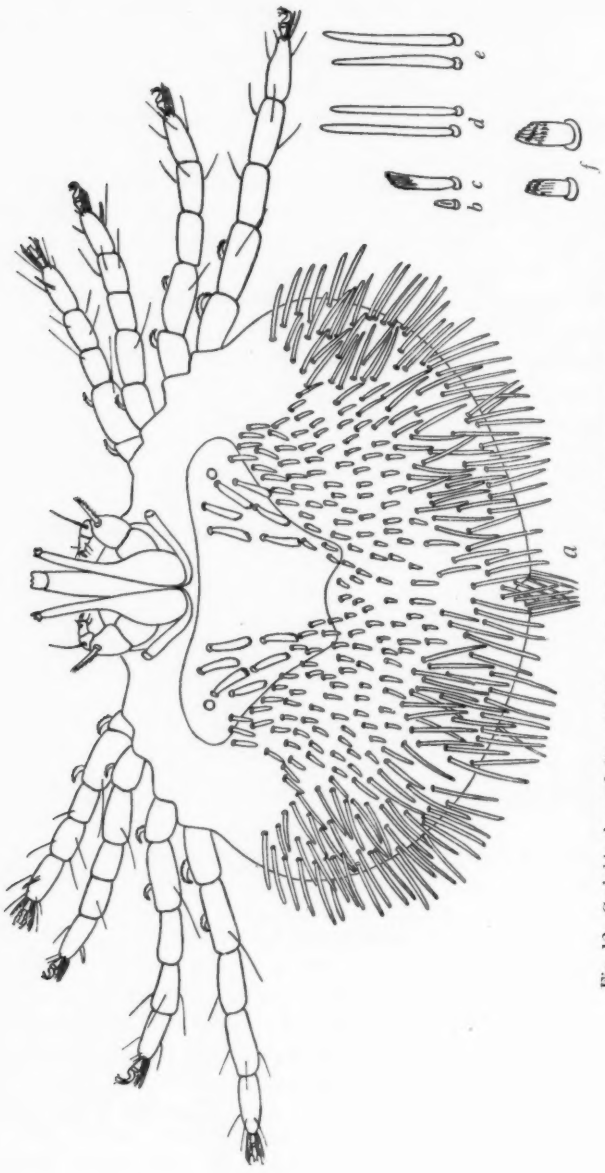


Fig. 12. *Geckobia homophalis*. Adult. *a*, dorsal view; *b*, hair from middle of the dorsum; *c*, anterior dorsal hair; *d*, peripheral hairs; *e*, ventral hairs; *f*, coxal spurs.

segment II of leg III provided with an incrassate hair; the incrassate hairs of segments I and II plumose along their whole length. Coxal spurs stout, subtriangular, and weakly plumose apically (Fig. 13 *e*).

Nymph and *larva* as in generic description and Fig. 2.

Dimensions. Adult: length of body 0·5 mm., breadth 0·56 mm. Nymph: length of body 0·28 mm., breadth 0·23 mm. Larva: length of body 0·2–0·24 mm., breadth 0·19–0·25 mm.

Other records. From the same host at Cape Town.

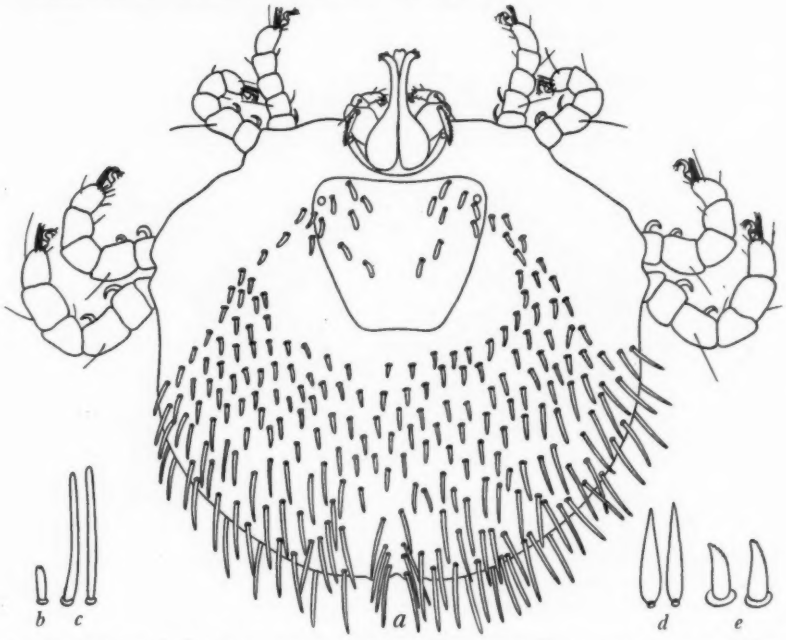


Fig. 13. *Geckobia phyllodactyli.* Adult. *a*, dorsal view; *b*, anterior dorsal hair; *c*, peripheral hairs; *d*, ventral hairs; *e*, coxal spurs.

Geckobia transvaalensis n.sp.

(Fig. 14)

Types. Eight specimens from *Lygodactylus capensis capensis*, Eastern Transvaal.

Body broader than long. Dorsal surface as in Fig. 14 *a*, the whole area thickly covered with fine hairs. Dorsal scute with two hairs at the side which are much longer than any of the remaining hairs (Fig. 14 *d*); hairs on the middle of the body small, subtriangular (Fig. 14 *b*); peripheral hairs long, fine, moderately plumose (Fig. 14 *e*). Ventral surface with long, slender, lanceolate hairs (Fig. 14 *f*), not as strongly plumose as the peripheral hairs of the dorsal surface.

Mouth-parts normal.

Legs normal, equal-sized. Coxal spurs (Fig. 14 *g*) short, plumose in their apical halves; the incrassate hair of segment I of leg IV (Fig. 14 *h*) large, plumose along its entire length.

Dimensions. Length of body 0·31 mm., breadth 0·42 mm.

Other records. From *Pachydactylus capensis affinis*, Plumtree, Southern Rhodesia; *P. capensis capensis*, Kimberley and Vryburg; *P. capensis tigrinus* Zoutpansberg; *Chondrodactylus angulifer*, Kanus Siding, South-West Africa. Two doubtful records from *Pachydactylus punctatus punctatus*, and *P. capensis formosus*, localities unknown.

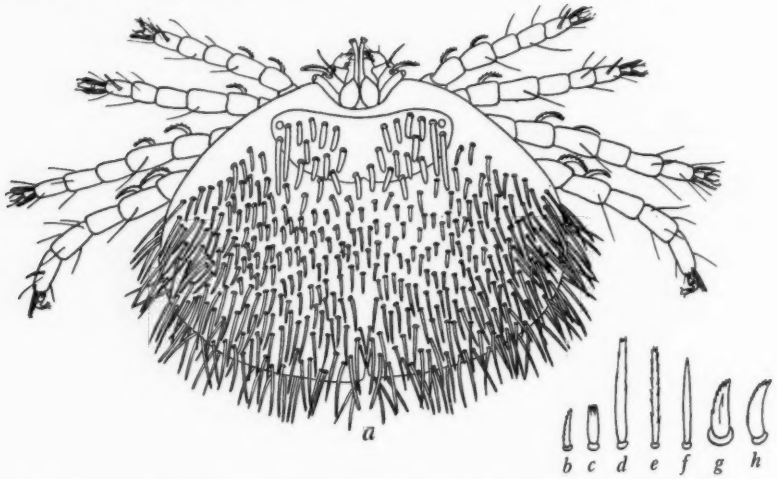


Fig. 14. *Geckobia transvaalensis.* Adult. *a*, dorsal view; *b*, hair from the middle of the dorsum; *c*, anterior dorsal hair; *d*, lateral hair of the dorsal scute; *e*, peripheral hair; *f*, ventral hair; *g*, coxal spur; *h*, incrassate hair on segment I of leg IV.

Geckobia karrooica n.sp.

(Fig. 15)

Types. Twenty-one specimens from *Oedura karrooica wilmoti*, Tarkastad.

Body about as long as broad or a little broader than long. Dorsal surface of body as in Fig. 15 *a*, the hairs on the middle of the body small (Fig. 15 *b*), those on the scute longer and slightly plumose (Fig. 15 *c*); peripheral hairs cylindrical, smooth, and distinctly longer and narrower than those on the dorsal scute (Fig. 15 *d*). Ventral surface covered with hairs as in Fig. 15 *e*; these are of uniform size and slightly plumose.

Mouth-parts rather deeply sunk in a concavity of the anterior margin of the body, the swollen bases of the chelicerae almost touching the anterior border of the scute.

Legs of uniform thickness, the posterior pair a little longer than the anterior

pair; otherwise normal; the incrassate plumose hair on the first segment of leg IV as in Fig. 15*f*. Coxal spurs much longer than broad, plumose at their apices (Fig. 15 *g*).

Dimensions. Length of body 0·42 mm., breadth 0·5 mm.

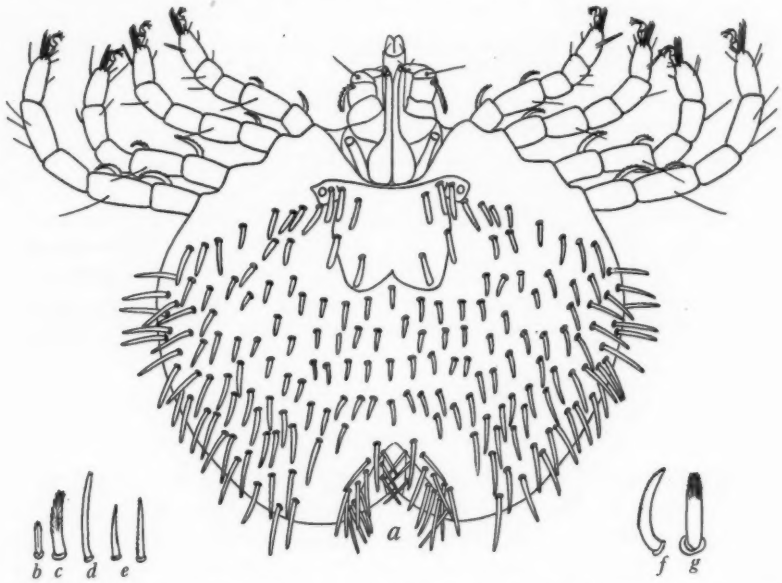


Fig. 15. *Geckobia karrooica.* Adult. *a* dorsal view; *b*, hair from the middle of the dorsum; *c*, anterior dorsal hair; *d*, peripheral hair; *e*, ventral hairs; *f*, incrassate hair on segment I of leg IV; *g*, coxal spur.

Key to the South African species of Geckobia

1. Ventral surface of body with distinctly flattened scale-like hairs, these not much longer than broad and quite unlike any hairs of the dorsal surface (Fig. 3*d*) ... **2**
   Ventral surface of body with long, slender, subcylindrical hairs resembling the peripheral hairs of the dorsal surface (Fig. 12*e*) **3**

2. Posterior legs longer and stouter than the anterior legs ... ... **australis**
   Legs equal-sized ... ... **ovambica**

3. Second segment of palps with a long, setiform, simple hair ... ... **tasmani**
   Second segment of palps with a short, incrassate plumose hair **4**

4. Dorsal surface with hairs of uniform size and shape, these usually stout, fairly short and cylindrical (Fig. 6*a*) **5**
   Dorsal surface with hairs differing strongly in form and size (Fig. 12*a*) **7**

5. Segment III of leg IV with an incrassate hair ... ... **oedurae**
   Segment III of leg IV without an incrassate hair **6**

6. Segment II of leg III with an incrassate hair ... ... **natalensis**
   Segment II of leg III without an incrassate hair ... ... **rhoptropi**

7. Mouth-parts long and slender, apical segment of palp several times longer than wide (Fig. 10*a*) ... ... **pachydactyli**
   Mouth-parts short and stout, apical segment not much longer than wide (Fig. 9*a*) **8**

8. Body subquadrate, the anterior legs well separated from the posterior ones ... **phyllodactyli**
   Body not subquadrate, the anterior and posterior legs close together **9**

9. Segment II of leg II and segment III of leg IV with a plumose hair **hewitti**
   These segments without a plumose hair **10**

10. The incrassate plumose hair on segment II of leg III absent ... ... **namaquensis**
    The incrassate plumose hair on segment II of leg III present **11**

11. Peripheral hairs of dorsal surface distinctly plumose **12**
    Peripheral hairs of dorsal surface smooth **13**

12. Dorsal scute with two lateral hairs almost twice as long as the remaining hairs (Fig. 14*a*) ... ... **transvaalensis**
    Dorsal scute without such hairs ... ... **hemidactyli**

13. Dorsal surface with numerous hairs varying considerably in size ... **homopholis**
    Dorsal surface with fewer hairs not varying greatly in size ... ... **karrooica**

III. MITES PARASITIC ON SOUTH AFRICAN AGAMIDAE

Genus *Pterygosoma* Peters

Adult. Dorsal surface of body with a patch of serrated hairs anteriorly on each side of the median line; posterior and lateral margins of the body with a row of either flattened paddle-shaped hairs, or pointed cylindrical hairs, or both; hairs absent from the middle of the dorsum; dorsal scute absent, eyes not discernible. Anal field situated on the dorsal surface of the body or partly dorsal and partly ventral, the arrangement of the anal hairs differing in the various species or groups of species. Ventral surface with three weak pairs of flattened hairs or spines. Mouth-parts covered basally by integument, not wholly exposed on the dorsal surface, segment II of the palpi always armed dorsally with a stout spine, never a plumose hair. Legs without flattened hairs on any of the segments, segment II of all legs with a stout, serrated, spiniform hair; coxae spined as in Fig. 16, Hirst, *J. Linn. Soc.* (*Zool.*), **36**, 196; anterior common coxa with a distal spine below each insertion of trochanters I and II, a third basal spine either at the base of the common coxa or a little way from it; posterior common coxa with a distal spine near the insertion of trochanter III and a basal spine. Body large, much wider than long.

Nymph much as in adult. Dorsal surface as in Fig. 16 *a* (*P. bedfordi*); posterior median opening not visible from above. Mouth-parts and legs as in adult. Ventral surface with three pairs of small spines; a small posterior median opening seen from below, armed with a pair of minute spines and flanked on each side by a flattened hair. Coxae seen from below armed as in adult.

Larva. Dorsal surface as in Fig. 16 *b* (*P. bedfordi*), eyes fairly large and clearly visible; mouth-parts and legs as in Fig. 16 *b*. Ventral surface with only one pair of long simple spines in the middle line near the posterior margin of the body; no posterior opening visible. Anterior common coxa with two spines, posterior coxa with one spine.

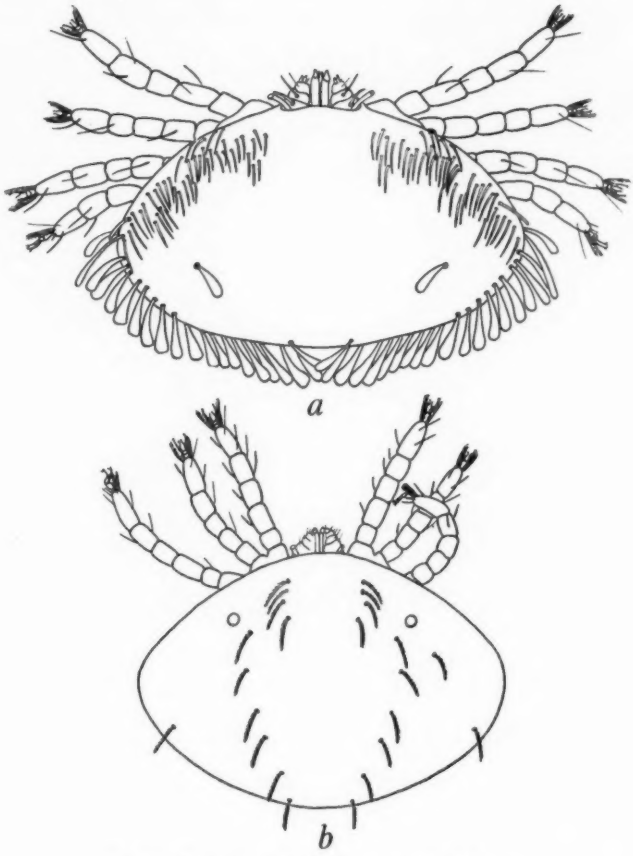


Fig. 16. *Pterygosoma bedfordi*. *a*, nymph; *b*, larva.

"Atra" *group*

Pterygosoma melanum Hirst

1926. Hirst, *J. Linn. Soc.* (*Zool.*), **36**, 196, Fig. 16.

The anal field situated on the dorsal surface and resembling that of *P. longipalpe* (Fig. 19 *a*). Mouth-parts long and slender, closely resembling those of *P. longipalpe* (Fig. 19 *b*).

Recorded on *Agama atra atra* from the following localities:

Cape Province: Cradock, Beaufort West; Kimberley; Fourteen Streams; Victoria West; van Rhynsdorp; Hanover, Phillipstown; Garies, Namaqualand; Hermanus; St James, Table Mountain, and Cape Point, Cape Peninsula.

Transvaal: Rustenburg; Pretoria; between Rustenburg and Pretoria; Rosslyn; Maquassie, near Wolmaranstad.

Bechuanaland: Vryburg.

Basutoland: Maseru.

South-West Africa: Luderitzbucht.

From *Agama anchietae knobelii*, Aus, South-West Africa.

This species, like its host, seems to be easily the most widely distributed of the Agamid *Pterygosoma* group. Nearly all the lizards examined were parasitised. It adheres rather loosely to the host, the whole body being exposed and not even partly covered by a scale. It is almost always attached near the lateral skin folds of the neck, in the arm-pits, or in the ear opening at the sides of the tympanum; it is rarely found on the legs or tail. The mites seem to be symmetrically disposed on each side of the body; thus if two mites are attached to the tympanum or three in the axilla of the one side, the same numbers are almost always found in corresponding positions of the opposite side. This arrangement was noted in so many lizards as to make it seem more than a mere coincidence.

The mite can be easily distinguished from other species of *Pterygosoma* by its robust appearance and oval shape, the width being less pronounced than in other species.

Pterygosoma hirsti n.sp.

(Fig. 17)

Types. Twenty specimens from *Agama atra atra*, Vondeling, Cape Province.

Body. Dorsal surface as in Fig. 17 *a*, differing from *Pterygosoma melanum* in only two respects, the 2–3 most anterior of the peripheral hairs being long and pointed, and the insertions of the peripheral hairs as they approach the anal field taking an inward curve, not following the margin of the body. Periphery with an average of nineteen hairs on each side (16–21 counted in six specimens), all inserted on the dorsal margin of the body. Anal field of the same general type as *melanum* and *longipalpe*; while in these two species the four pairs of racquet-shaped hairs and the pair of posterior spines is wholly visible on the dorsal surface, only two pairs of rounded hairs can be seen from above in *hirsti* (Fig. 17 *c*), the remaining two and the pair of posterior spines being inserted on the ventral surface (Fig. 17 *d*). This arrangement is constant throughout the seven specimens mounted and must be considered of specific value. In some cases pressure in mounting the specimens brings about an alteration in the boundary of the posterior margin of the body, the relations of the structures surrounding the anal field being then disturbed. I feel confident, however, that in this case no distortion has occurred and that

Figs. 17 *c* and *d* represent the normal dorsal and ventral aspects of the anal field in this species. Ventral surface with three pairs of spines, their positions similar to those figured by Hirst (*loc. cit.* Fig. 16, p. 196), these positions being constant in all species of *Pterygosoma* found on South African Agamidae.

Mouth-parts short and stout, never protruding far beyond the anterior margin of the body, as in Fig. 17 *b*, the structures seen from above through

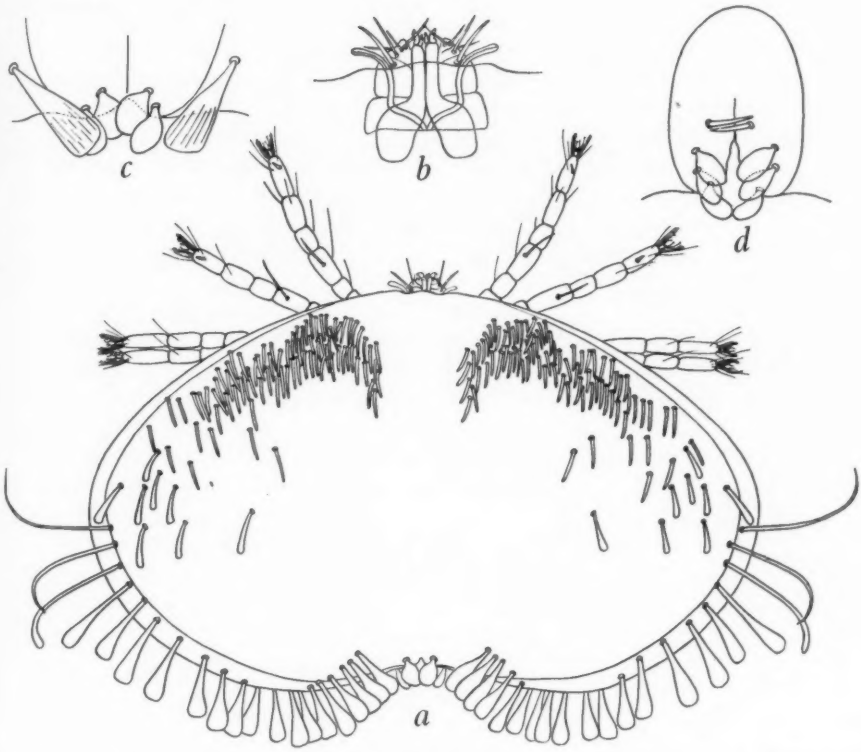


Fig. 17. *Pterygosoma hirsti*. Adult. *a*, dorsal view; *b*, mouth-parts, dorsal view; *c*, anal field, dorsal view; *d*, anal field, ventral view.

the transparent skin, chelicerae with the distal narrowed portion only a little longer than the swollen basal portion; palpi short, the segments subglobose and indistinctly defined, segment II with a stout spine (not as stout as in *melanum*), tapering distally.

Legs as in Fig. 17 *a*.

Dimensions. Length of body 0·75 mm., breadth 1·4 mm.

P. longipalpe was taken from the same individual host.

Pterygosoma bedfordi n.sp.

(Fig. 18)

Types. Seventy specimens from *Agama atra atra*, Hermanus, Cape Province.

Body. Dorsal surface as in Fig. 18 *a*, differing from the two preceding species in having an average of twenty-five (23–28 counted in six specimens) paddle-shaped hairs along each side of the periphery, these hairs duplicated

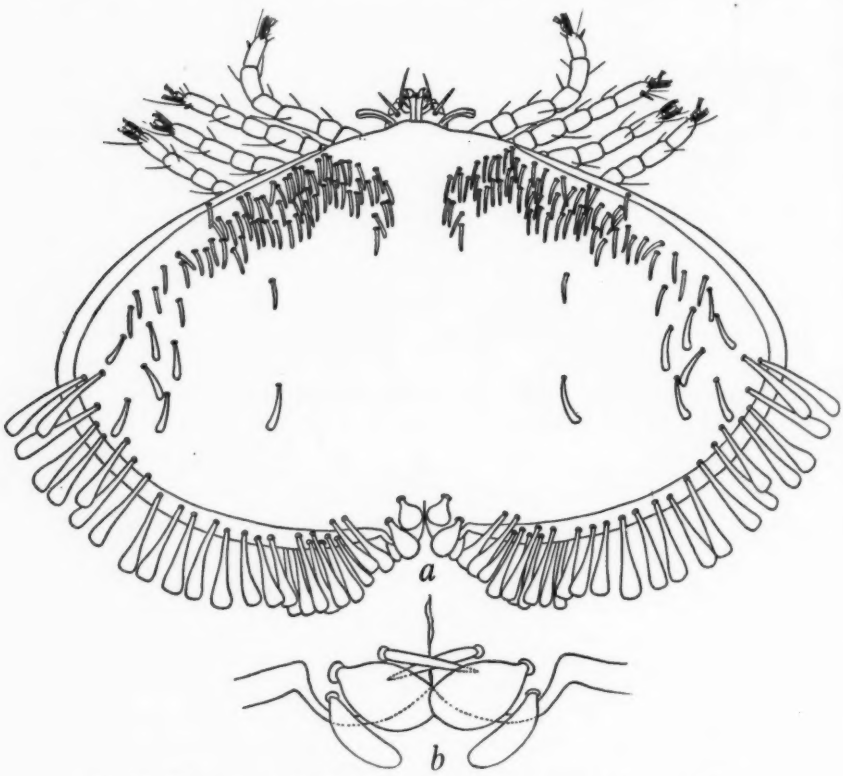


Fig. 18. *Pterygosoma bedfordi.* Adult. *a*, dorsal view; *b*, anal field, ventral view.

and forming a cluster on each side of the anal field. Anal field as in Fig. 18 *a* seen from above, Fig. 18 *b* seen from below, with two pairs of racquet-shaped hairs inserted on the edge of the body and more laterally to the pair of spines than in either *hirsti* or *melanum*.

Mouth-parts stout and short, as in *hirsti*.

Legs as in Fig. 18 *a*.

Nymph and *larva* as in generic description, p. 20, and Fig. 16.

Dimensions. Adult: length of body 0·63 mm., breadth 1·1 mm. Nymph: length of body 0·24 mm., breadth 0·4 mm. Larva: length of body 0·25 mm., breadth 0·36 mm.

P. melanum was taken from the same individual host.

Pterygosoma longipalpe n.sp.

(Fig. 19)

Types. Ten specimens from *Agama atra atra*, Namaqualand.

Body. Dorsal surface as in Fig. 19 *a*, differing from *hirsti* and *bedfordi* in having an average of thirty-one peripheral hairs on each side (28–35 counted in seven specimens), these not forming a cluster on each side of the anal field, fairly long and slender and of uniform shape and size; except for a few near the lateral angles, and some others near the anal field, all are inserted close to the margin on the ventral surface; anal field as in Fig. 19 *a*, all the hairs inserted on the dorsal surface, the posterior pair of spines inserted either dorsally, or at the edge, or ventrally. Ventral surface with three pairs of spines.

Mouth-parts as in Fig. 19 *b* seen from above, the component structures seen through the transparent skin, resembling those of *melanum* in being very long and slender; chelicerae especially long, the distal narrowed portion about twice as long as the swollen basal portion; palps long and slender, segment II with a very stout spine tapering to a fine point, this spine about twice as thick at its base as the spine on segment III but a little shorter than this spine. Fig. 19 *b* drawn to the same scale as the mouth-parts of *hirsti* (Fig. 17 *b*).

Legs as in Fig. 19 *a*.

Dimensions. Length of body 0·67 mm., breadth 1·2 mm.

Other records. From *Agama atra atra*, Vondeling, from *A. hispida aculeata*, Murraysburg.

"Hispida" *group*

Pterygosoma agamae Peters

1849. Peters, W., *S.B. Ges. naturf. Fr. Berlin.*
1926. Hirst, S., *J. Linn. Soc.* (*Zool.*), **36**, 194, Fig. 14.

Taken on *Agama kirkii* from Mazoe, Chishawasha, and Zimbabwe, Rhodesia; from specimens labelled *Agama mossambica*, Mozambique.

Pterygosoma aculeatum n.sp.

(Fig. 20)

Types. Twenty-four specimens from *Agama hispida aculeata*, Strydenburg, Cape Province.

Body. Dorsal surface as in Fig. 20, periphery on each side with an average of thirty-five pointed hairs (34–38 counted in six specimens), the great majority inserted on the dorsal margin; a few of these hairs very slightly flattened at

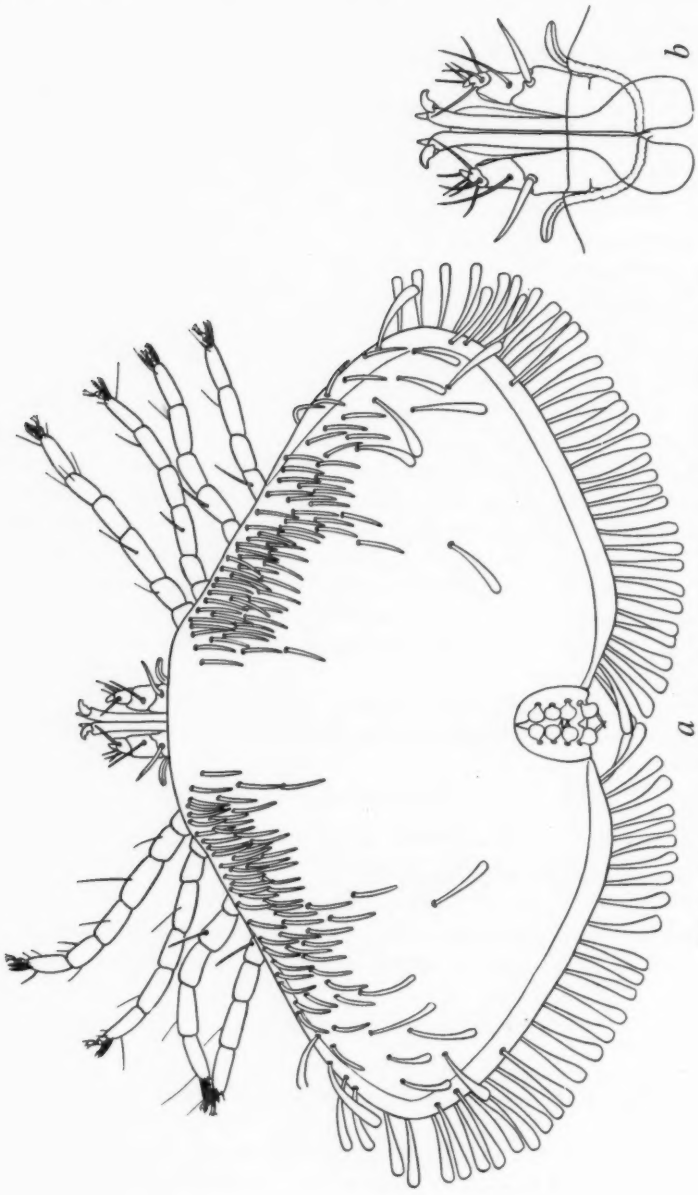


Fig. 19. *Pterygosoma longipalpe*. Adult. *a*, dorsal view; *b*, mouth-parts, dorsal view.

their apices. Anal field situated on the dorsal surface, with five more or less equal-sized hairs on each side, the two anterior ones flattened in their distal halves but not strongly so, the remaining three more or less cylindrical.

Mouth-parts of the long and slender type, the narrowed distal portion of the chelicerae about $1\frac{3}{4}$ times as long as the basal swollen portion; spine on segment II of palps about $1\frac{1}{2}$ times as thick at its base as the spine on segment III.

Legs as in Fig. 20.

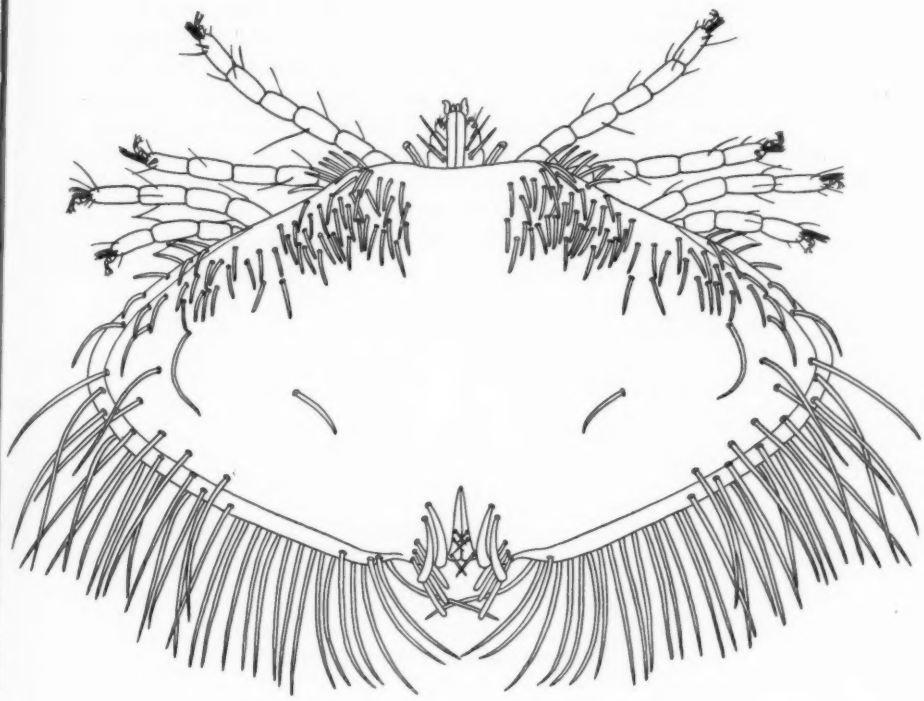


Fig. 20. *Pterygosoma aculeatum*. Adult, dorsal view.

Dimensions. Length of body 0·63 mm., breadth 1·18 mm.

Other records. From *Agama hispida aculeata*, Kaotwe, Central Kalahari; Kakamas; Kimberley; Fourteen Streams. From *A. hispida distanti*, Pretoria (four separate records); Chishawasha, Rhodesia.

This species is allied to *agamae*, but differs from it in the shape of the peripheral hairs, and in the two anterior hairs of the anal tuft being considerably narrower. It also differs from *neumanni* in the details of these two structures.

Pterygosoma triangulare n.sp.

(Fig. 21)

Types. Thirty specimens from *Agama hispida brachyura*, Bitterfontein, Namaqualand.

Body of adult, fully-fed specimens, large and bluntly triangular in shape, much more so than is represented in Fig. 21 which is drawn from a mounted specimen. Dorsal surface as in Fig. 21, periphery on each side with an average of twenty-eight hairs (26–32 counted in six specimens), those on each side of the anal field duplicated to form a thick cluster of 10–15 hairs; between this cluster and the group of anal hairs on each side, a space quite free of hairs; the cluster of hairs on each side of the anal field very slightly expanded at their apices and frayed, the remaining hairs pointed; the great majority of the hairs inserted on the dorsal periphery. Anal field situated on the dorsal surface, with 5–7 hairs on each side (usually five) the two anterior ones much more flattened than the remaining three, of which the most anterior is subparallel, the two others pointed; the two anterior hairs differing from those of *aculeatum* in being only half the length of the three posterior hairs. Ventral surface with three pairs of very slender spines.

Mouth-parts short and stout, the palpi with indistinctly defined subglobose segments, II with a stout spine twice as thick at its base but shorter than the spine on segment III.

Legs as in Fig. 21.

Dimensions. Length of body 0·63 mm., breadth 1·5 mm.

Other records. From *Agama hispida brachyura*, unknown locality; from *A. hispida hispida*, Darling; Port Nolloth.

This species resembles *neumanni* in the shape of the peripheral hairs but differs from it in the number and arrangement of these hairs, the number and shape of the anal hairs, and the mouth-parts.

Pterygosoma transvaalense n.sp.

(Fig. 22)

Types. Seven specimens from *Agama atricollis*, Mariepskop, Eastern Transvaal.

Body small. Dorsal surface as in Fig. 22, periphery with an average of thirteen hairs (11–15 counted in three specimens), these expanded and striated apically but not strongly so; all the peripheral hairs inserted on the dorsal surface. Anal field with seven hairs on each side, five or six of these inserted on the dorsal surface, one or two on the ventral surface; all these hairs, especially the two anterior ones, more or less expanded distally, the expanded portion striated; in addition a pair of strong spines at the posterior apex of the anal region ventrally. Ventral surface with the usual three pairs of fine spines.

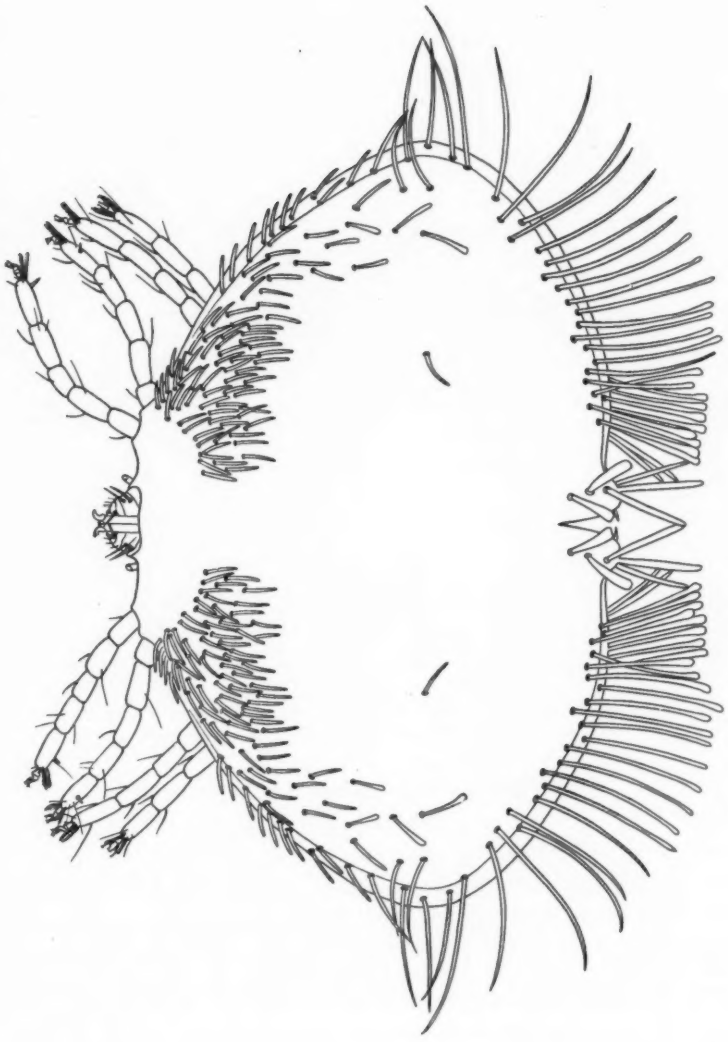


Fig. 21. *Pterygosoma triangulare*. Adult, dorsal view.

Mouth-parts short and stout, the segments of the palps subglobose, spined as in the previous species, chelicerae with the distal narrowed portion only 1¼ times as long as the swollen basal portion.

Legs as in Fig. 22.

Dimensions. Length of body 0·48 mm., breadth 0·9 mm.

Other records. From the same host, Weenen, Natal.

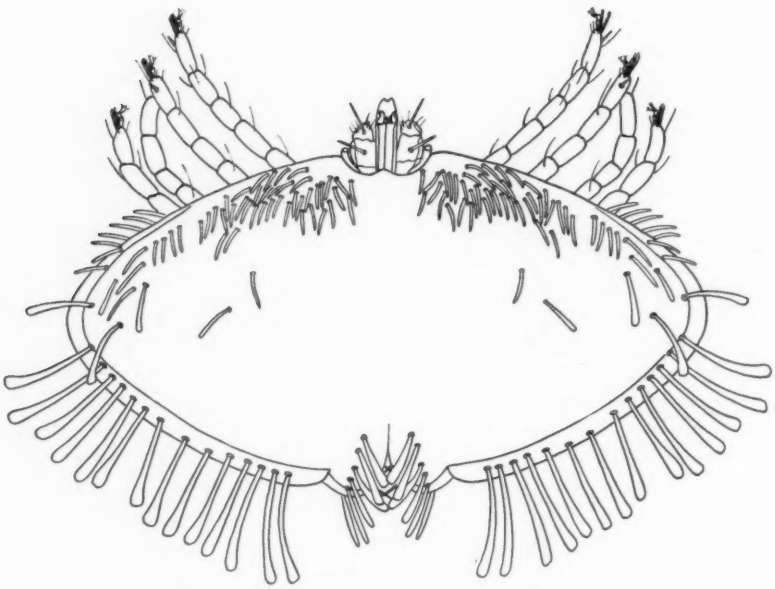


Fig. 22. *Pterygosoma transvaalense.* Adult, dorsal view.

Pterygosoma armatum n.sp.

(Fig. 23)

Types. Seven specimens from *Agama hispida armata*, Weenen Natal.

Body. Dorsal surface as in Fig. 23, periphery with an average of twenty-two hairs (21–26 counted in three specimens), these all inserted on the dorsal periphery, all apically expanded and striated except 2–3 of the lateral ones. Anal field as in Fig 23, with only four pairs of hairs, the two anterior ones strongly flattened and rounded, shaped more or less like those of the *atra* group of species, the two remaining hairs inserted on the posterior edge of the anal field, cylindrical or more or less flattened but not racquet-shaped; in addition an unusually strong pair of spines inserted on the ventral surface at a level corresponding to half-way between the insertions of the two dorsal hairs. Ventral surface with three pairs of spines.

Mouth-parts short and stout as in the preceding species.

Legs as in Fig. 23.

Dimensions. Length of body 0·62 mm., breadth 1·1 mm.

The arrangement and structure of the anal hairs seem to be almost exactly intermediate between those displayed by the species of the *atra* and *hispida* groups.

Fig. 23. *Pterygosoma armatum*. Adult, dorsal view.

Key to the South African species of Pterygosoma

1. Anal field with four pairs of round racquet-shaped hairs 2
   Anal field armed with 4–7 pairs of much longer paddle-shaped or cylindrical hairs ... 5
2. Mouth-parts long and slender (Fig. 19*b*) 3
   Mouth-parts short and stout (Fig. 17*b*) 4
3. Periphery with about thirty-one slender hairs ... ... **longipalpe**
   Periphery with about twenty shorter hairs ... ... **melanum**
4. Periphery with about twenty-five hairs, all expanded distally ... ... **bedfordi**
   Periphery with about nineteen hairs, the three anterior ones pointed distally **hirsti**
5. Periphery with at most fifteen hairs ... ... **transvaalense**
   Periphery with 22–35 hairs 6

6. Anal field with four pairs of hairs, the two anterior pairs racquet-shaped (Fig. 23) **armatum**
   Anal field with 4–7 pairs of hairs, the two anterior pairs not racquet-shaped (Fig. 20) 7

7. Periphery with about thirty-five hairs, these all pointed distally ... **aculeatum**
   Periphery with 28–32 hairs, not all pointed distally 8

8. Hairs at the lateral angles of the body pointed, anterior anal hairs much shorter than the posterior ones ... ... **triangulare**
   All peripheral hairs slightly flattened distally, anterior anal hairs not much shorter than the posterior ones ... ... **agamae**

IV. GENERAL DISCUSSION

(*a*) The correlation of parasite and host in the family Geckonidae

It is unfortunate that the material is insufficient, in the case of the Geckonidae, for a definite pronouncement on a number of points. It must also be remembered that in this large family many of the rarer species and subspecies are described from, and are still represented by, two, three, or a single specimen. Changes in nomenclature and differences in the opinions of workers as to the value to be assigned to certain characters have resulted in errors in the identification of some of the Museum material; an unfortunate result has been that the same form is in some cases labelled with different names in the several Museums. This, however, would apply more to subspecific than to specific differences, and on the whole the identifications may be regarded as correct, or nearly so.

As will be seen from the list of South African Geckonidae and their parasites on p. 37, of the sixty-nine species and subspecies contained therein, about twelve have not been examined for parasites, while still others (about twenty-four) have been examined in insufficient numbers; it has been considered unwise in cases where less than six specimens have been examined to make any definite assertion as to whether a lizard species is parasitised or not; furthermore, in such cases the few specimens available have often been collected in one locality, and experience has proved that a number of individuals from the same locality are either all positive or all negative for parasites.

In drawing conclusions from the correlation between parasite and host in this family, therefore, it is necessary to restrict these conclusions to generic groups, while it is unsafe as yet to do so in the case of specific groups. When some species have been found to be negative, although the majority of species are positive in a given genus, it may be said that the genus as a whole harbours parasites and that many of the negative species will prove to be positive when adequate material has been handled. Of such genera *Pachydactylus* affords a good example.

The majority of the South African Geckonid genera are rupicolous in habit, living on or in the crevices of rocks, or under stones; as in the case of Zonurid

lizards they are all definitely parasitised. Genera such as *Phyllodactylus*, *Oedura*, *Hemidactylus*, *Lygodactylus*, *Homopholis*, *Pachydactylus* and *Rhoptropus* are all partly or completely rupicolous and all are parasitised. There can be no doubt that the great majority of lizards, in the western half of South Africa at least, are rupicolous, as distinguished from an arboreal or burrowing mode of life. Such widespread genera as *Hemidactylus*, *Lygodactylus* and *Pachydactylus* are probably partly arboreal, or arboreal in one locality and rupicolous in another. *Homopholis* is probably arboreal in the eastern half of South Africa. Cott (1934) has reported *Pachydactylus bibronii* as being arboreal in Eastern Africa; but it is certainly the most striking example of a rock-living gecko in Namaqualand.

Of the remaining genera *Narudasia* has not been examined in adequate numbers; those genera of which ample material has been examined, and which have no parasites, viz. *Palmatogecko*, *Ptenopus*, and *Colopus*, are all sand-living burrowers. *Chondrodactylus angulifer* is an exception, but in this case, of twenty-five specimens examined only a single one was parasitised by *Geckobia transvaalensis*, a species of scale mite which had been taken on *Lygodactylus capensis* and several species of *Pachydactylus*. It seems likely therefore that *Geckobia transvaalensis* is a parasite with considerable powers of adaptation to different hosts, and the specimens found on *Chondrodactylus* should be regarded as stragglers.

The difficulty of correlating parasite and host in species rather than genera of Geckonids is borne out by the fact that in some species of lizards, *e.g. Hemidactylus tasmani* and *Lygodactylus capensis*, different and unrelated parasite species occur. In other Geckonids, *e.g. Phyllodactylus porphyreus*, *Lygodactylus capensis*, *Homopholis wahlbergii*, and *Pachydactylus bibronii*, the mite parasite is obviously limited to a single host species.

With regard to the parasite fauna itself, consisting of fourteen species within a single genus, *Geckobia*, the various forms can be easily separated from each other. The characters of these forms are so distinct and constant that in some of the species systematists might be led into creating new genera, or at any rate subgenera, to receive them. For example, *Geckobia pachydactyli* is different from any known species of the genus, the tarsi of the palps being greatly elongated. Any subdivision of the genus, however, would, the writer considers, be an error. Throughout the large number of known species the essential generic characters, such as the presence of numerically constant coxal spurs, are alone enough to unite the parasites of the Geckonid lizards into a group differing conspicuously from all other prostigmatic scale mites.

(*b*) THE CORRELATION OF PARASITE AND HOST IN THE FAMILY AGAMIDAE

The array of Agamid material in the South African museums is an impressive one, and all the known forms have been examined. The family is in the first place a small and compact one, consisting as it does of a single genus with about fifteen distinguishable species and subspecies. These forms with

their areas of distribution are all well known, and it is unlikely that many more South African Agamidae remain to be described. The difficulty facing the systematist in this family is more that of defining the subspecific forms and separating them morphologically and geographically from other closely allied subspecies. Though the species themselves are clear-cut and distinct from each other, some of them cover an enormous geographical area and these have tended to develop a number of subspecies with distributional areas which overlap each other; in the intermediate localities these subspecies tend to intergrade so that precise distributional boundaries for each form are difficult to prescribe. Such a species is *Agama hispida*, with its varietal forms. This group, together with the groups of varietal forms centering round *A. atra* and *A. anchietae*, were systematically revised by Boulenger and Power in 1921, and many of the forms treated as species by the older authors were reduced to varietal rank. This view has been followed here, being considered as most nearly approaching the actual systematic relationships of Agamid lizards. In addition to these three species or groups of forms there appear in the South African region two other well-defined species, *atricollis*, *planiceps*, and two closely related East African species, *kirkii* and *mossambica*; the latter are regarded in this discussion as a single unit, the "*mossambica*" group. There are thus in the region considered seven species, which, with the subspecies centering round them, it may be convenient to term "groups".[1] Of these seven species only *planiceps* is without scale parasites, though a fair number of specimens from different localities have been examined. It should be noted that in this species, the lepidosis is fine and close-fitting, with few rough or spiny scales; there is but little imbrication. In *atricollis* scale mites seem to be extremely rare, but in all the other species they are abundant, especially in the *atra* and *hispida* groups.

With regard to the relationships of scale mites of the genus *Pterygosoma*, the main consideration in distinguishing them has been the arrangement, shape, and number of the hairs associated with the region which has been called the "anal field". The same procedure has been adopted as that used for *Zonurobia* in a previous paper. The number of peripheral body hairs and the structure of the mouth-parts are much less reliable than the characters of the anal field; the number and shape of the peripheral hairs may vary considerably, and the structure of the mouth-parts be completely different in closely allied parasites; these allied forms, as will be seen from the systematic part of the paper, may even occur on the same individual lizard. On the basis of the hair characters of the anal field, the Agamid scale mites can be divided into two primary groups, an *atra* group and a *hispida* group. In the *atra* group the hairs of the anal field always consist of four pairs, and are round and racquet-shaped, or sometimes wider than long; at the base of the four hairs is a pair of spines. In the *hispida* group the anal hairs are nearly always more than four pairs, always much longer than wide, and never round, being

[1] *Mossambica* and *kirkii* being regarded as a single unit, there are thus six "groups".

more often cylindrical; the two anterior hairs are usually flattened and paddle-shaped. A less reliable distinction is the presence of strongly flattened peripheral hairs in the *atra* group, while those of the *hispida* group tend to be long, slender, and pointed. This character, however, cannot be regarded as wholly satisfactory, for in *Pterygosoma agamae* and *P. transvaalense* the tips of the hairs tend to be more or less expanded. The *hispida* group also includes *P. neumanni*, the parasite of *Agama colonorum* from Senegal, which is, however, not included in the scope of this paper. Between these groups and apparently connecting them, is a single species, *armatum*, which on the characters of the anal field would not fit into either group, but to a certain extent resembles both of them.

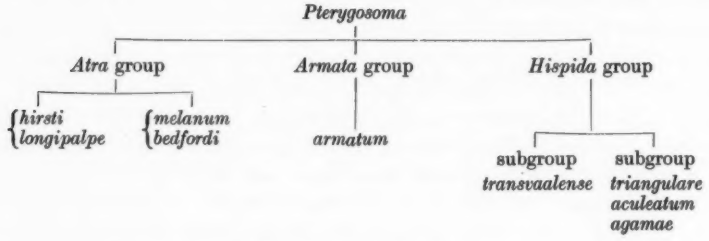
The atra *group.* This group consists of four species all occurring on the typical form of *Agama atra*, with one record from *A. anchietae knobelii*. These species have been found occurring in pairs on the same individual lizard, *bedfordi* and *melanum* forming one pair, *hirsti* and *longipalpe* another. The members of both pairs differ from each other in the same characters; each pair consists of one form with elongated mouth-parts and one with greatly shortened, rounded mouth-parts, the form with the shorter mouth-parts being also provided with 6–12 more hairs on each side than its partner. It seems fairly certain that these heteromorphic forms found on the same individual host (which have also been noted by Trägardh (1905) and Hirst (1926) for *Geckobia*) are forms which have different fixation areas. The type of *Pterygosoma* with slender mouth-parts, *e.g. melanum*, is found in adult specimens to be loosely attached to the skin on the side of the body where the scaling is finer; the body of the mite is completely exposed. In the case of the type with short mouth-parts, the mite is usually attached beneath the large imbricating scales of the dorsal or ventral surfaces and the body is largely hidden by the scale. The two types can be very easily distinguished by their mouth-parts in the nymphal stage. At present no explanation can be offered for the presence of four species of parasite on one species of host. It may eventually be found that the host species of *longipalpe* and *hirsti* is not typical *atra* but *Agama atra rudis* or another undescribed variety. The whole group of four species must be considered, on the evidence of the character of the anal field, to be closely related.

The hispida *group.* This group consists of four species (five if the species from *Agama colonorum* is included). In this group the three species *agamae*, *triangulare*, and *aculeatum*, form a closely related subgroup in respect both of the anal and peripheral hairs; *transvaalense* differs rather markedly in having more numerous and rather differently shaped anal hairs, while the number of peripheral hairs is much reduced (12–15). Of the three remaining species both *aculeatum* and *triangulare* differ from *agamae* in the shape of the anterior anal hairs; *triangulare* has a superficial resemblance to *neumanni* in the shape of the peripheral hairs, but its real affinities are probably with *aculeatum* to which it seems to stand in much the same relation that *bedfordi* does to *melanum*

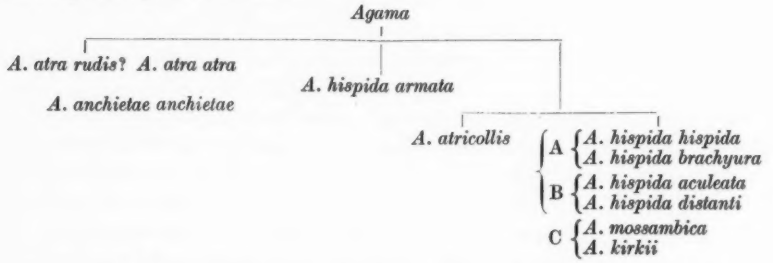
in the *atra* group. It should be noted that there are here the same two types of mouth-parts as are found in the *atra* group, *aculeatum* having long, and *triangulare* short segments, in this structure. A further parallel exists in the cluster of duplicated hairs on each side of the anal field which is found in *triangulare* and *bedfordi*.

The armata *group.* The scale parasite of *Agama hispida armata* is here provisionally treated as forming a distinct group in view of the fact that it agrees with neither the *atra* nor the *hispida* groups. In the single species, *armatum*, the two anterior hairs of the anal field resemble those of the *atra* group, while the two posterior ones are formed like those of the *hispida* group. The peripheral body hairs are both spatulate and pointed.

These groups and subgroups of parasites can be represented as follows:



If the name of the host species is substituted for the parasite species the following arrangement results:



From this table it will be seen that the parasites found on four of the forms of *hispida*, viz. *typica*, *brachyura*, *aculeata*, and *distanti*, are closely related; the *brachyura* form of *hispida* shares the same species of scale mite with the typical form, while *aculeata* and *distanti* share another parasite species closely related to that of *brachyura-typica*. Boulenger and Power (1921) have noted intergrading forms of *distanti* and *aculeata* while the same is most probably true of *brachyura* and the typical *hispida*. The two latter forms would more often occupy the same areas in the south-western parts of the Cape Province, while the two former ones do actually have large distributional areas in common, more northwards than those of the *brachyura-typica* combination. The presence of one species parasitic on the northern group of *hispida* forms and a separate

species on the southern group seems to point to a primary division into a northern *aculeata-distanti* group and a southern *typica-brachyura* group, before these groups themselves started to subdivide into their two component subspecies. There is no evidence that there has been crossing between mite species from morphologically and geographically allied species. This crossing does not seem to occur in the Zonurid parasites, but may have happened in the Agamidae, where such rigid isolation of species as occurs in Zonurid lizards, is unknown. The peculiar intermediate position of the species of mite found on *Agama hispida armata* might be accounted for if it was considered to be a hybrid of mites of the *atra* and *hispida* groups. The geographical distribution of this subspecies would enable it to come into contact with lizards of both groups. Finally, the relationships of its mite parasites point to the fact that the *hispida* group is far more closely allied to the *mossambica* group (*A. mossambica*, *A. kirkii*), than to *A. atricollis*.

V. LIST OF MITES PARASITIC ON SOUTH AFRICAN GECKONIDAE AND AGAMIDAE

The numbers of individual lizards examined are placed in brackets after the names of those species from which no parasites have been obtained. Species without these numbers have not been examined.

Family GECKONIDAE

Host species	Parasite species
Chondrodactylus angulifer	*Geckobia transvaalensis*
Ptenopus garrulus garrulus (45)	—
P. garrulus maculatus	—
Palmatogecko rangei (10)	—
Phyllodactylus lineatus lineatus (5)	—
P. lineatus essexi	—
P. peringueyi	—
P. porphyreus	*G. phyllodactyli*
Oedura africana (2)	—
O. amatolica (2)	—
O. karrooica (1)	—
O. karrooica wilmoti	*G. karrooica*
O. langi (3)	—
O. marleyi (1)	—
O. nivaria	—
O. pondolia (1)	—
O. pondolia multiporis (1)	—
O. tembulica (10)	—
O. transvaalica (6)	—
O. transvaalica platyceps	*G. oedurae*
Hemidactylus mabouia	*G. hemidactyli*
H. tasmani	*G. tasmani*
	G. hemidactyli
Lygodactylus capensis capensis	*G. australis*
	G. natalensis
	G. transvaalensis

Host species	Parasite species
Lygodactylus capensis ngamiensis	*Geckobia transvaalensis*
L. bradfieldi	*G. rhoptropi*
L. lawrencei (1)	—
L. ocellatus (13)	—
L. picturatus chobiensis	—
L. stevensoni (2)	—
Homopholis wahlbergii	*G. homopholis*
Pachydactylus amoenus	—
P. austeni (1)	—
P. bibronii bibronii	*G. pachydactyli*
P. bibronii laevigatus (15)	—
P. bibronii stellatus	—
P. capensis affinis	*G. transvaalensis*
P. capensis capensis (=*P. leopardinus*)	*G. transvaalensis*
P. capensis fasciatus (3)	—
P. capensis formosus (=*P. capensis mentomarginatus*)	*G. transvaalensis* (doubtful record)
P. capensis gariesensis	*G. namaquensis*
P. capensis levyi (=*P. capensis o'shaughnessyi*) (3)	—
P. capensis oculatus	—
P. capensis tigrinus	*G. transvaalensis*
P. capensis vansoni (3)	—
P. capensis weberi (1)	—
P. latirostris (1)	—
P. montanus (1)	—
P. maculatus maculatus	*G. hewitti*
P. maculatus albomarginatus	*G. hewitti*
P. mariquensis (20)	—
P. mentalis (2)	—
P. namaquensis	*G. namaquensis*
P. ocellatus (20)	—
P. punctatus punctatus	*G. transvaalensis* (doubtful record)
P. punctatus brunnthaleri	—
P. punctatus bicolor (3)	—
P. punctatus langi (4)	—
P. purcelli (5) (=*P. pardus*)	—
P. rugosus (12)	—
P. scutatus (3)	—
P. serval (1)	—
Colopus wahlbergii	—
C. kalaharicus (4)	—
Rhoptropus afer (2)	—
R. barnardi	*G. ovambica*
R. braconnieri	—
R. bradfieldi (8)	—
R. ocellatus	*G. rhoptropi*
Narudasia festiva (6)	—

Family AGAMIDAE[1]

Host species	Parasite species
Agama atra atra	*Pterygosoma melanum*
	P. hirsti
	P. bedfordi
	P. longipalpe
A. atra rudis (4)	—
A. anchietae anchietae	*P. melanum* (1 example)
A. anchietae knobelii (3)	—
A. anchietae methueni (9)	—
A. atricollis	*P. transvaalense*
A. hispida hispida	*P. triangulare*
A. hispida armata	*P. armatum*
A. hispida aculeata	*P. aculeatum*
A. hispida brachyura	*P. triangulare*
A. hispida distanti	*P. aculeatum*
A. hispida makarikarika (1)	—
A. kirkii	*P. agamae*
A. mossambica	*P. agamae*
A. planiceps (20)	—

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[1] The variety *Agama atra holubi* Bocage, regarded as an uncertain variety by Boulenger and Power (1921), is not included in this list.

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A STUDY OF THE HAPLORCHINAE (LOOSS 1899) POCHE 1926 (TREMATODA: HETEROPHYIDAE)[1]

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(With 12 Figures in the Text)

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I. Introduction

Introductory remarks

Members of the genus *Haplorchis* Looss 1899 are quite common in cats and dogs from Kwangtung Province. We have already reported such findings last year from Canton (Chen, 1934*a*, 1934*b*), and further examinations of cats from Yeung-Kong were also positive, but because of inadequate descriptions of certain important parts of these flukes by previous authors an attempt to identify all of them to species became very difficult. Furthermore, since these flukes are so minute and since the descriptions are in many cases inadequate there is a great possibility that two or more authors might describe several different species under one name or same species under two or more different names. So the task of identifying these flukes was left until the present, after the writer had obtained the materials of most of the species described by various authors for comparison.

Previous investigators (Faust and Nishigori, 1926; Witenberg, 1929; Katsuta, 1932*a*, 1932*b*; and Gohar, 1934) have emphasised too much the size of eggs, position of ovary, and morphology in general as specific differences, and have emphasised only slightly chitinous armature on the ventral sucker. From our own experience we have found that variations are very great in the same species as to the points just mentioned, while the only constant character

[1] Contribution from the Biological Laboratory, Lingnan University, Canton, China. The investigation was supported by a grant from the Rockefeller Foundation in China.

is the form of the acetabular armature, which is not only constant among forms found here but also among those found in Palestine and Formosa. With these facts in mind and also with the knowledge of the minuteness of these flukes we will see that any attempt to identify these worms by their general form and structures other than the acetabular spines or hooks may lead one to confusion, which is further intensified by the commonly mixed infections of two or three species in one host. There is a great deal of evidence that some previous investigators have got the species confused. The present study is partly aimed, therefore, to elucidate the detailed structures of the acetabular armature, so that we may be able to discuss the validity of the various species described for this genus.

Acknowledgments

The writer wishes to acknowledge his gratefulness to Prof. E. C. Faust of Tulane University, U.S.A., Prof. G. Witenberg of Hebrew University, Jerusalem, and particularly to Prof. S. Yokogawa of the Government Medical College of Formosa, Taihoku, for their kindness in loaning, for this study, their specimens, most of which are types and cotypes. Without the co-operation of these men the present study would be most difficult. The writer wishes also to thank Dr W. A. Riley, University of Minnesota, U.S.A., for securing certain inaccessible literature for this work.

Material

A large number of *Haplorchis taichui*, *H. pumilio* and *H. yokogawai* were obtained from Canton and Yeung-kong cats and dogs. Type specimens of *Monorchotrema taihokui*, *M. microrchia* and *M. yokogawai* from Dr Yokogawa, cotypes of *Monorchotrema taihokui* and *M. taichui* from Dr Faust, and *Haplorchis taichui* and *H. pumilio* from Dr Witenberg, were all studied and carefully compared.

II. Historical review and taxonomic position of *Haplorchis* and its various species

The subfamily Haplorchinae (Looss 1899) Poche 1926 of Heterophyidae was established by Looss to include two genera, *Haplorchis* and *Galactosomum*. The latter genus was later transferred to the Heterophyinae by Witenberg (1929). Two species belonging to *Haplorchis* were known, namely, *H. cahirinus* (Looss 1896) and *H. pumilio* (Looss 1896). In 1924 Nishigori established the subfamily Monorchotreminae for the reception of a new genus *Monorchotrema* and two new species of this genus, *M. taihokui* and *M. taichui*, being apparently unaware of Looss' species. This subfamily is characterised by having "a single large testis, and the genital sucker fused in part with the ventral sucker, surrounded by a half circlet of genital hooklets".[1] The life cycle was

[1] These are the original words used by Faust and Nishigori (1926) for the diagnosis of the subfamily. But in the description of the two species of the genus *Monorchotrema*, they used the word "spine". The use of two different words of different meanings to describe the same structure is very misleading.

subsequently worked out by Faust and Nishigori (1926). Witenberg (1929) in Palestine reported the same flukes from cats and dogs, but intimated that *Haplorchis* and *Monorchotrema* might be identical. Later (1930) he definitely stated that *Monorchotrema* should fall into synonymy with *Haplorchis*. After his transferring the genus *Galactosomum* from Haplorchinae to Heterophyinae and including the genus *Monorchotrema* in Haplorchinae he characterised the latter as follows (1929): "*Heterophyidae* with flattened but not dilated anterior part of the body; circumoral spines absent; one testis; ovary and seminal receptacle situated in front of testis."

Katsuta (1932*a*, 1932*b*) described two more species under the genus *Monorchotrema*, namely *M. yokogawai* and *M. microrchia*, from dogs and cats in Formosa, arguing that although the genus *Haplorchis* may be identical with *Monorchotrema*, yet it was first properly described under the latter name. In the present author's opinion Looss' description of *H. pumilio*, particularly in regard to the armature of the ventral sucker, is very careful and entirely adequate for a diagnosis of the genus.

Tubangui (1933) added another species, namely *Haplorchis anguillarum*, to the list. This member has two testes instead of one, and Tubangui argued that "the presence of double testes is here considered of specific rather than of generic importance...". If we accept the diagnosis of the subfamily Haplorchinae, which appears to be of a satisfactory basis for the classification, we will certainly not accept Tubangui's contention.

Gohar (1934) described a species, *Haplorchis milvi*, from the Egyptian kites, and advocated the idea that *Haplorchis* is distinct from *Monorchotrema*. In the absence of the type specimen of *Haplorchis* he proposed to characterise this genus as follows: "Testicule et ovaire globuleux, parfois un peu ovales, disposés obliquement, l'ovaire étant en avant du testicule et à droite", and, on the other hand, he went on characterising *Monorchotrema*, of which *M. taihokui* is the type species, in the following words: "Testicule et ovaire globuleux placés l'un derrière l'autre, l'ovaire en avant, tous deux étant toujours médian." On the basis of the above distinctions Gohar broke up *Haplorchis* into two genera and placed, in the genus *Haplorchis*, *H. pumilio*, *Monorchotrema taichui* and *H. milvi*, and, in the genus *Monorchotrema*, *M. taihokui*, *M. microrchia*, *M. yokogawai* and *Haplorchis cahirinus*.

In so doing Gohar has overlooked the fact that there are great variations as to the shape of the testis and ovary and their relative positions among individuals of the same species. Furthermore, the hinder part of an *Haplorchis* tends to be cylindrical, and the relative position of the different organs, including the testis and ovary, therefore, depend a great deal on how the worm is placed during mounting. We have seen considerable variations among individuals of the same species in our own collections as to the relative positions of the two organs. We, therefore, do not agree with Gohar's classification.

After this review it seems logical that the subfamily Monorchotreminae and its type genus *Monorchotrema* can no longer stand. The species described

under this name should be transferred to the genus *Haplorchis* and subfamily Haplorchinae in accordance with the law of priority. Furthermore, Gohar's judgment on the differentiation of the two genera in the subfamily Haplorchinae is unsound and cannot be accepted.

III. MORPHOLOGY AND CLASSIFICATION OF HAPLORCHINAE

Family HETEROPHYIDAE Odhner 1914

Diagnosis. Very small Trematodes with more or less oval body, posterior portion generally widened. Integument covered with scale-like spines. Oral and ventral sucker present, and in addition there is often a genital sucker near acetabulum. Pharynx present, oesophagus relatively long, intestinal caeca simple and of varying lengths. Reproductive system in posterior part of body, one to two testes, cirrus pouch absent, ovaries in front of testes, seminal receptacle well developed. Adults live in the intestine of mammals and birds. Early larval stages are found in snails and metacercariae, so far as known, encyst in fish.

Subfamily HAPLORCHINAE Poche 1926

Diagnosis. Anterior part of body somewhat flattened. Ovary in front of testis; the latter single and located near posterior end of body, genital sucker fused in part with ventral sucker.

Type genus. Haplorchis Looss.

Genus **Haplorchis** Looss 1899

Diagnosis. Body oval or somewhat elongated; prepharynx present, oesophagus long; seminal receptacle to right of ovary; seminal vesicle in several parts; vitellaria in posterior part of body; uterine coils behind ventro-genital-sucker complex; genital sac slightly to one side; anterior end of ventral sucker bears on its surface chitinous armature; excretory bladder Y-shaped.

Type species. Haplorchis pumilio (Looss 1896). Other valid species are *H. taichui, H. yokogawai* and *H. milvi.*

Haplorchis pumilio (Looss 1896) (Figs. 1–4)

Synonym. *Monorchotrema taihokui.*

The Trematode is somewhat flattened dorso-ventrally at the anterior half of the body with the two extremities rounded off gradually. Body covered with rows of short backwardly directed flat scales, becoming less conspicuous towards the posterior extremity.

Average body size of nineteen worms is 0·295 × 0·636 mm., width 0·165–0·36 mm., length 0·32–0·95 mm. Oral sucker terminal, width 0·062 mm., length 0·053 mm. (average of eight worms). Acetabulum slightly to the right of the body. It is armed at the anterior region by an almost complete row of hooks, each of which is typically an arrow-like structure with a triangular spear point towards the outer end and with one to three, or sometimes more,

lateral hooks near the middle and at the base of the shaft (Figs. 2 and 4). These lateral branches are stout and pointed, while some shafts tend to be slender, and in extreme cases a shaft may entirely disappear towards the middle so that the two extremities of the chitinous structure become disconnected. It is somewhat difficult to count the exact number of hooks present but in all well-flattened specimens thirty-five to thirty-seven hooks can be observed. Not all the hooks are of the same length, the longest being 0·011 mm. and the shortest 0·003 mm. or much less. The area surrounded by the circle of hooks is about 0·062 × 0·047 mm.

The genital pore is about 0·026 mm. in diameter. It opens into a sinus, the walls of which converge together like a funnel into a small opening which is guarded by a few chitinous rodlets. This funnel-like structure is very muscular and is known as the genital sucker. Its distal end is fused with the ventral sucker, the two together constituting a ventro-genital-sucker complex. Into the genital sinus open two ducts, one of which is the ejaculatory duct and the other the vagina, both being abundantly supplied with muscle fibres.

The mouth opening leads into a short prepharynx, about 0·015 mm. long, followed by a pharynx, 0·028 × 0·029 mm. The slender oesophagus is about 0·10 mm. long. Bifurcation of the caeca begins slightly anterior to the middle of the body in front of the ventro-genital-sucker complex. The caeca are three or four times as large as the oesophagus and extend to as far as a little distance in front of the anterior border of the testis.

The large and smooth testis is situated at the posterior part of the body. It may be spherical or slightly elongated, diameter about 0·12 mm. A single duct from its anterior part leads to the seminal vesicle situated horizontally, or more often obliquely, on the left side of the body behind the ventro-genital-sucker complex. It is often divided into two constrictions, a large anterior portion, and smaller posterior portion. The anterior portion of the seminal vesicle is narrowed down to form a tubular prostate duct which continues forward as an ejaculatory duct abundantly supplied with muscle fibres and opens into the genital sinus.

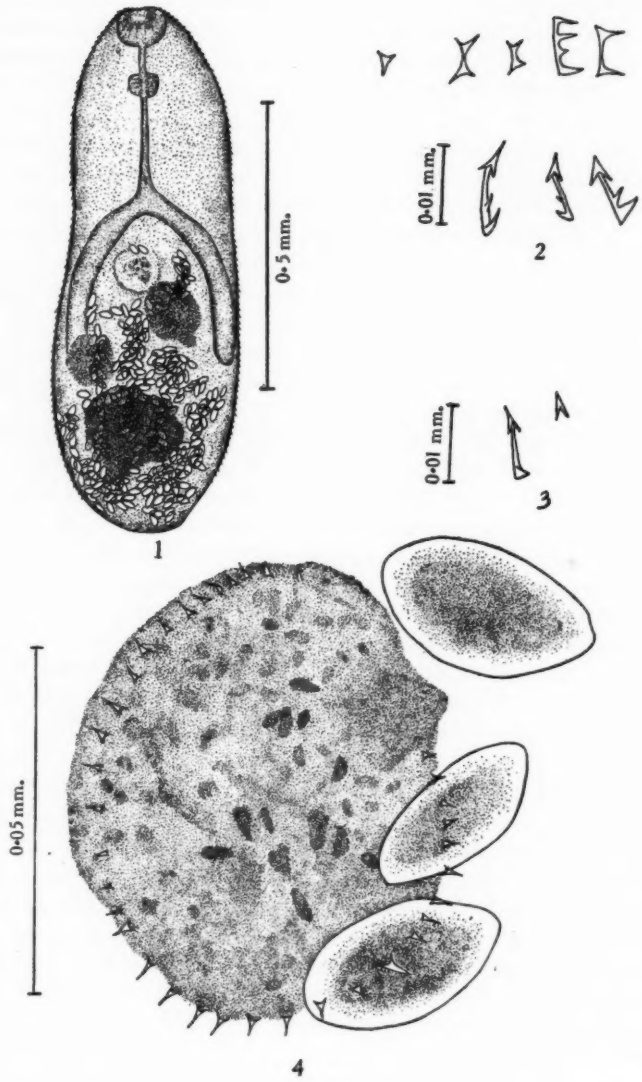
Legends to Figs. 1–4.

Fig. 1. *Haplorchi pumilio.* Whole mount.

Fig. 2. *H. pumilio*, acetabular hooks much enlarged. Note the triangular spear head and branches from the shaft, and particularly the increasing complexity of the hook from the small to the full size one. The four figures from the right side on the first row are shown in lateral views.

Fig. 3. Acetabular hooks of "*Monorchotrema taihokui*", from a type specimen sent by Dr Yokogawa. The species is now considered as a synonym of *Haplorchis pumilio*. The size and structure of hooks are quite different from those in the original description of "*Monorchotrema taihokui*", but very similar to the description of *Haplorchis pumilio* by Looss, and to our specimens.

Fig. 4. *Haplorchis pumilio*, acetabular hooks *in situ*. Note that only the triangular spear heads are shown, the shafts with their branches being imbedded in the tissue and not visible unless carefully focused. It is believed that this peculiar condition explains partly why some previous workers called them spines and gave much shorter measurements. Three eggs are seen at the side.



Figs. 1–4.

All the drawings were made with the aid of a camera lucida. Figs. 1, 4, and 5–9 were drawn by Miss Lee Ping I of this laboratory. No attempt is made to draw the detailed structures of an entire animal in Figs. 1, 5 and 9.

Directly or obliquely toward the right in front of the testis, and often separated by a space is the ovary which is spherical or slightly elongated, and smooth in outline. It measures about 0·07 × 0·046 mm. On the right side between the testis and the ovary is the dorsal seminal receptacle which measures about 0·08 × 0·06 mm. The size varies considerably and it tends to become spherical. The uterus passes from the ovary to the left, makes several loops around the lower right corner of the testis, turns forward to the right side, making several loops again, and, crossing the region between the ventro-genital-sucker complex and the ovary, turns upward and to the right, joining the vagina. Vitellaria are in small follicles occupying the region behind the seminal vesicle. Eggs average 30 × 17·6 μ, length 24·92–32·04 μ and width 14·28–17·8 μ, characteristically possessing a minute filament at the posterior pole.

Host. Dogs, cats, rabbits, rats, mice, guinea-pigs, men and birds (*Nycticorax nycticorax*, *Milvus migrans*, *M. parasiticus* and *Pelecanus onocrotalus*).

Location. Intestine.

Geographical distribution. Egypt, Formosa, Palestine and South China.

Comments. In the original description of *Haplorchis pumilio* by Looss the acetabular hooks were well described, but do not agree in detail with those of the species found here. These hooks, the exact number of which is not mentioned, are arranged in an incomplete circle near the genital sinus, and each being 3 μ long, slightly curved, pointed towards one extremity, truncated towards the other and provided with three very fine lateral branches. Our present specimens agree with everything mentioned except the length, and the number of lateral branches. The disagreements can easily be due to the host and geographical differences, or individual differences of the specimens, and certainly cannot be significant enough to be considered as of specific importance unless there are some other constant structural differences. It seems, therefore, reasonable to place the present specimens in *H. pumilio*.

Faust and Nishigori (1926) in their study of the morphology and life history of *H. taihokui* (Nishigori 1924) have described the acetabular hooks as "...spines in the form of chitinous rodlets...generally measuring 1·7 μ to 2·5 μ in length....It is estimated that there are between thirty-six to forty-two of them". The description is quite different from that given by Looss, but considering the great similarity in the general description of the two forms, one wonders whether it may be the same species as *H. pumilio* and that the difference in the description of the chitinous structures may be due only to careless observations. Through the kindness of Dr Yokogawa we had the opportunity of examining a type specimen, originally called *Monorchotrema taihokui* described in detail by Faust and Nishigori. We have found that the so-called rodlets are really the same kind of peculiar hooks with branches as found in our own specimens and are therefore very similar to those described by Looss, and that the longest hooks are about 10 μ (Fig. 3). In this particular specimen the hooks are very close together with one another instead of being

some distance apart like those in most of our own specimens. This, we believe, is due to individual differences or the peculiar position of the acetabulum.

We have also examined a cotype specimen of the same species sent by Dr Faust, and found that the situation is unexpectedly different from the original description by Faust and Nishigori and the type specimen sent by Dr Yokogawa. Here we find many more than forty-two tiny spines (not hooks), less than 1 μ long, all over the face of the ventral sucker. Dr Faust in his personal communication to us stated that the original authors had apparently overlooked these spines, but since the latter were not found together with the acetabular hooks in this particular specimen and in other specimens collected locally we believe that the cotype specimen belongs to *Haplorchis yokogawai*.

Based on the original description of the acetabular armature and other structures of "*Monorchotrema taihokui*", Witenberg (1929) identified some *Haplorchis* from Palestine as *Monorchotrema taihokui* but later, we believe correctly, as *Haplorchis pumilio*. Nevertheless, he fell into the same mistake in overlooking the peculiar hooks and describing them as "...a row of about 40 very minute spines". A specimen sent by Dr Witenberg shows that the acetabular armature is almost the same as described by Looss and exactly the same as our own specimens.

The difficulty of giving exact descriptions of these chitinous structures lies undoubtedly in their peculiar shape and in their proximal part being often buried in the tissue, so that very often only the triangular spear points at the distal end were observed. The best methods of seeing them distinctly, according to our experience, are (1) to dissect the ventro-genital-sucker complex out, or (2) to let a dying specimen well flattened out by the pressure of a cover-glass in a fresh mount. The latter method is less tedious and easier.

Haplorchis taichui (Nishigori, 1924) (Figs. 5–8)

Synonym. *Monorchotrema microrchia*.

The worm is flattened and slightly narrowed at the anterior half, but broad and almost cylindrical at the posterior half, with both extremities gradually rounded off. Body covered with scales, more conspicuous at the anterior half and diminished in size and number toward the posterior extremity. The average size of nine worms is 0·66 × 0·32 mm., length 0·58–0·76 mm., width 0·27–0·42 mm. Oral sucker terminal and about 0·06 mm. diameter. Ventral sucker buried in the parenchyma slightly to the right of the body and closely associated with the genital sucker, forming a ventro-genital-sucker complex. Anterior end of ventral sucker armed with fourteen to twenty, more often fifteen, spines arranged like a fan placed diagonally toward the lower right corner of the sucker (Fig. 6). The largest spines, which are from 18–21 μ long, are at the middle of the fan and those toward the edge become shorter and more slender. Some may be as short as only 1 μ, although this is rare. The fan is 0·035–0·053 mm. across.

Prepharynx conspicuous, about 0·02 mm. long. Pharynx is 0·032 × 0·027 mm. Oesophagus slender about 0·09 mm. long. The two intestinal caeca are large and extend to the posterior end of the body. Testis large, 0·11 mm. long by 0·085 mm. wide. In front of it and often close together is the smaller ovary about 0·065 mm. diameter. To the right and somewhat posterior is a large

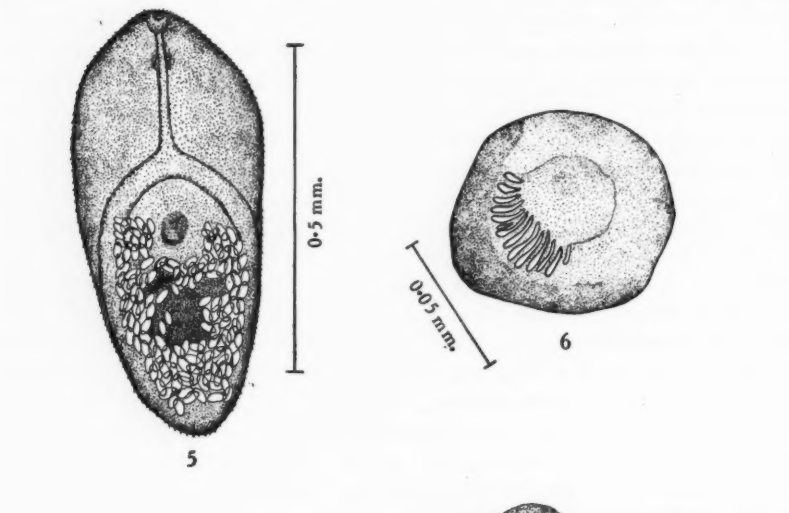


Fig. 5. *Haplorchis taichui*, whole mount.
Fig. 6. *H. taichui*, acetabular spines.
Fig. 7. *H. taichui*, acetabular spines, drawn from a cotype specimen sent by Dr Faust.
Fig. 8. Acetabular spines from a type specimen of "*Monorchotrema microrchia*" sent by Dr Yokogawa. The specific name is now considered as a synonym of *Haplorchis taichui*.

seminal receptacle 0·107 mm. diameter. Seminal vesicle divided into two parts, the posterior, smaller, 0·036 × 0·046 mm., the anterior one larger 0·07 × 0·09 mm. The two are distinct, having a constriction at the region of junction. They are obliquely placed on the left side of the body just below the ventral sucker. The anterior seminal vesicle is narrowed to form a tubular

prostate duct which continues forward as a short ejaculatory duct and opens into the genital sinus. The uterus contains numerous eggs, is much coiled, and after a sinuous course reaches the vagina. Vitellaria consist of small follicles located behind the seminal vesicle. Eggs small, with a minute filament on the posterior end. They are $29{\cdot}5 \times 15{\cdot}7\,\mu$ in average, length varying $27{\cdot}3$–$32\,\mu$, and width $14{\cdot}24$–$17{\cdot}8\,\mu$.

Host. Dogs, cats, birds (*Milvus migrans*), and experimentally in man.

Location. Intestine.

Geographical distribution. Formosa, Palestine, Egypt and South China.

Comments. The general morphology, together with the details of the acetabular armature of the Kwangtung material, is similar to the description of Faust and Nishigori (1926) for the Formosan species and Witenberg (1929) for the Palestine species. The Formosan species, however, is decidedly smaller.

Katsuta (1932*a*) described a new species under the name of *Monorchotrema microrchia*, from Formosa. The armature of the ventral sucker, according to him, consists of forty-six to forty-eight conspicuous radiating spines arranged much like a chrysanthemum flower. On examining one type specimen of *M. microrchia* which was kindly loaned to the writer by Dr Yokogawa, we found that on the ventral sucker there are only fourteen or fifteen spines arranged in the shape of a fan (Fig. 8) instead of having forty-six to forty-eight arranged like a chrysanthemum flower. In other words, it suggests that this species may fall into synonymy of *Haplorchis taichui* (Nishigori 1924). After this unexpected observation we wrote to Dr Yokogawa, who agreed with our observation by saying: "...I examined a specimen I had with me and found that the spines were not arranged in a complete chrysanthemum flower shape in the fixed specimen, while in the number I could see clearly up to 18–20."

However, Dr Yokogawa in the personal communication further stated that according to Dr Katsuta, who originally described this species, the spines were arranged clearly in a chrysanthemum flower shape in the fresh specimen of both the metacercaria and the adult, and that such pattern not seen in fixed specimens might be due to the special structure of the ventral sucker. If Dr Yokogawa's supposition were correct, it seems very strange that a complete circle of these spines should appear only in the fresh materials and not in the fixed specimens. Furthermore, the number and size of the spines and their arrangement agree very well with *H. taichui*. It does not seem possible that the other half of the spines could become lost or imbedded in the tissue. The fan-shaped arrangement is just as natural as that found in *H. taichui*, except that in this particular one Dr Yokogawa sent us it is found on the lower left corner of the ventral sucker instead of the lower right. Before there is any definite proof that *H. microrchia* is structurally different from *H. taichui*, we must consider the former as a synonym of the latter.

Haplorchis yokogawai (Katsuta 1932) (Figs. 9–12)

The worm is generally flattened dorso-ventrally at the anterior half, and is oval at both ends. The body 0·54–0·94 mm. long with an average size of 0·7 × 0·284 mm., and is armed with backwardly directed scale-like spines, either lying flatly upon the body or standing up obliquely at an angle. They gradually disappear near or at the posterior tip of the body.

Oral sucker terminal 0·065 × 0·062 mm. Ventral sucker buried in paren-

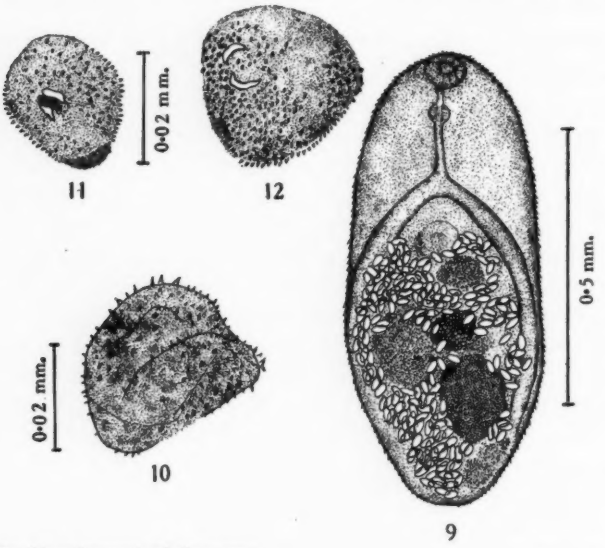


Fig. 9. *Haplorchis yokogawai*, whole mount.

Fig. 10. *H. yokogawai*, acetabular spines; size somewhat exaggerated. Note that the spines are generally distributed over the anterior end of the sucker.

Fig. 11. *H. yokogawai*, acetabular spines of another specimen; size somewhat exaggerated. Note the two relatively large chitinous structures near the middle. The visibility of the latter depends a great deal on the position of the sucker.

Fig. 12. *H. yokogawai*, acetabular spines from the type specimen sent by Dr Yokogawa, size somewhat exaggerated. Note that the distribution of the spines is quite different from the original description of the species, but more like that in our own species.

chyma and closely associated with the genital sucker at the middle of the body to form a ventro-genital-sucker complex. Anterior border of the ventral sucker is armed with numerous tiny spines, very seldom more than 1 μ long, uniformly or partially scattered on the surface, surrounded by a semicircular row of the same kind of spines which, because of regional differences, are often more conspicuous (Figs. 10 and 11). Excentrically on the spiny surface, or sometimes in the centre, there are usually two chitinous structures with an irregular margin of approximately 5 × 2 μ (Fig. 11), or there may be a bush of irregular spiny projections instead. These structures may not be seen in many specimens due to the peculiar position of the ventral sucker. They

ornament the genital orifice in the genital sinus. The size of the surface covered by the spines is about 0·026 mm. diameter when round, but very often it may tend to be semicircular, or even somewhat irregular.

Mouth opens into prepharynx about 0·027 mm. long. Pharynx 0·032 × 0·035 mm. Oesophagus 0·07–0·13 mm. long, intestinal caeca large, reaching the level of the posterior border of the testis.

Testis large, single, 0·142 × 0·119 mm., posterior to ovary. The two are often very close together. Directly or obliquely in front lies the smaller, round or oval ovary about 0·072 × 0·059 mm. Seminal receptacle 0·134 × 0·104 mm. to the right and somewhat posterior. Left of the ovary and anterior is the seminal vesicle which lies diagonally from left to right and often in two constrictions, anterior portion 0·07 × 0·053 mm., posterior portion 0·037 × 0·027 mm. A narrow prostate duct goes forward which continues as a short ejaculating duct joining the genital sinus. Uterus sinuous and contains numerous eggs. Vitellaria consist of small follicles, all situated behind seminal vesicle. Eggs oval, 28·48–30·26 μ long × 14·24–16·02 μ, often possess a minute filament at the posterior pole.

Host. Dogs, cats, and experimentally in man.

Location. Intestine.

Geographical distribution. Formosa, South China and (?) Egypt.

Comments. In his original description of this species, Katsuta characterised the armature of the ventral sucker as having seventy to seventy-four spines arranged in a horse-shoe form around the anterior end of the ventral sucker, each spine being 4·8–8 μ long × 0·8 μ. On examining a type specimen that Dr Yokogawa sent us we found that the condition of the armature of the ventral sucker is quite different from the original description but similar to the form just described (Fig. 12). Our identification is based, therefore, not on the original description but on the re-study of the type specimen.

The species *H. milvi*, described by Gohar (1934), is characterised by having sixty to seventy spines arranged in a crown ornamenting the genital orifice. This characterisation is insufficient, and in judging the disagreement already encountered it may possibly fall into synonymy with *H. yokogawai*. Decision concerning the matter can be made only when the type specimen is re-studied.

The difficulty as seen from these disagreements concerning the number and arrangement of the spines is apparently due to their minuteness and their peculiar position. It is not uncommon that we often observed only the spines on the outermost margin and it was not until after our attention had been directed to the structure for a while that we began to see spines scattered within.

IV. General discussion

We have noted in our previous works (Chen, 1934*a*, 1934*b*) that 13·5 per cent. of dogs examined were positive for *Haplorchis pumilio*, and that 42·1 per cent. of cats examined for *H. pumilio*, *H. yokogawai* and *H. taichui*. Of

the three species found in cats the last two constitute only a small percentage of the infection. *H. pumilio* is the most abundant form of the genus found in cats and dogs in Kwangtung Province.

One of the chief aims of this paper is to bring to light the fact that the only constant specific difference practical enough for diagnosis among the species of *Haplorchis* is the forms of armature on the ventral sucker. Other differences may help, but are in most cases not dependable. Table I is a

Table I. *Specific characters of the three species of* Haplorchis[1]

	H. taichui	*H. yokogawai*	*H. pumilio*
Size of body	0·58–0·76 long 0·27–0·42 wide	0·54–0·94 long 0·26–0·38 wide	0·32–0·95 long 0·165–0·36 wide
Oral sucker	0·06 dia.	0·063 dia.	0·053 long × 0·62 wide
Prepharynx	0·02 long	0·027 long	0·015 long
Pharynx	0·032 long 0·027 wide	0·035 long 0·032 wide	0·029 long 0·028 wide
Oesophagus	0·09 long	0·07–0·13 long	0·10 long
Intestinal caeca	Extend to posterior end of body	Extend to posterior end of body	Extend to near ant. border of testis
Acetabular armature	14–20 spines arranged like a fan	Numerous tiny spines generally distributed	Almost complete circle of 35–37 hooks
Seminal vesicle:			
Anterior	0·09 × 0·07	0·07 × 0·053	0·13 × 0·10
Posterior	0·046 × 0·036	0·037 × 0·027	0·055 × 0·035
Seminal receptacle	0·107 dia.	0·134 × 0·104	0·08 × 0·06
Ovary	0·065 dia.	0·072 × 0·059	0·07 × 0·046
Testis	0·11 × 0·085	0·142 × 0·119	0·12 dia.
Eggs	0·0273–0·032 × 0·014–0·018	0·0285–0·03 × 0·0142–0·016	0·0249–0·032 × 0·0143–0·0178

summary of measurements and characteristics of some of the more readily observed parts of the three Haplorchids described in this paper. It is unnecessary to emphasise that the measurements vary a great deal according to whether they are taken when the worms are alive or dead; if dead, how killed and preserved; and finally the state of contraction. So by size of various parts alone without a detailed description of the acetabular armature, specific diagnosis becomes very difficult.

Except *H. taichui*, the spines of which were very well described and figured by Faust and Nishigori (1926) and Witenberg (1929), and *H. pumilio*, the hooks of which were well described by Looss (1896 *a*), the acetabular armature of the other two species has been either insufficiently or inaccurately described for specific diagnosis. This accounts for part of the reason why there is such a confusion concerning the different species in this genus. The confusion is made worse by the commonly mixed infections of more than one species in the same host, so that it is possible that some author is unaware that he is dealing with two or more species. If we admit this fact, then it seems necessary

[1] Measurements are all in mm. Eggs were measured in living worms under cover-glasses, all other measurements being from materials fixed in mercuric chloride mixture.

that the life history of some of these forms which has already been worked out must be very carefully checked over.

Another constant character, which may divide the species of *Haplorchis* just described into two groups is the length of the intestinal caeca. In *H. pumilio* they are short and extend to as far as near the anterior border of the testis. In the original description Looss stated that he had never seen how far the caeca extended. Gohar found a species in Egypt which he called *H. pumilio* and which was characterised by having the caeca extending to the posterior end of the body. This characteristic, together with his description of the acetabular armature, makes one wonder whether or not he was merely dealing with *H. yokogawai* or an entirely distinct species instead of with *H. pumilio*. In *H. yokogawai* and *H. taichui* the caeca reach the posterior end of the body.

From the foregoing descriptions and discussions of the different species of *Haplorchis* we have noted that the validity of the described species is narrowed down to:

(1) *H. pumilio* (Looss 1896),

(2) *H. taichui* (Nishigori 1924),

(3) *H. yokogawai* (Katsuta 1932),

(4) *H. milvi* Gohar 1934,

making a total of four valid species. Although the type specimen of *H. pumilio* has been claimed to be lost, yet the description of the acetabular armature is in such a detail that there remains very little doubt as to its specific standing. Efforts were made to obtain *H. milvi* for comparison, but for some unavoidable reason without any success. Nevertheless, from the description it appears to be quite possible that this species may fall into synonymy with *H. yokogawai*. There are still two other species which have been assigned to this genus, namely, *H. cahirinus* (Looss 1896) and *H. anguillarum* Tubangui 1933. Indeed, as Witenberg (1930) has pointed out, the long prepharynx and the more anterior position of the testis together with its being found in a fish should be sufficient to exclude *H. cahirinus* from this group, at least, in our opinion, until the type specimen may be redescribed. In the case of *H. anguillarum* there is still less ground for its being placed in this genus or the subfamily, the reason for which has already been given in the earlier part of this paper.

A key is included in the following by which it is hoped that the different species of *Haplorchis* known may be correctly identified.

Key to the Species of Haplorchis

1. Anterior end of the ventral sucker armed with peculiar hooks, arranged in an almost complete circle, longest being about 11 μ, each with one to several lateral branches; intestinal caeca reaching near anterior border of testis ... ... **H. pumilio**
   Anterior end of the ventral sucker not armed with hooks but with spines; intestinal caeca reaching posterior end of body **2**

2. Spines large, longest ones may reach 20 μ, ranging between eleven and twenty in number, arranged like a fan ... ... **H. taichui**
Spines small, numerous, either distributed in a circle or over the anterior end of acetabulum **3**

3. Spines are generally distributed over anterior end of acetabulum, very numerous **H. yokogawai**
Spines arranged in a crown ornamenting genital orifice ... ... **H. milvi**

V. Summary

1. A detailed historical account of the subfamily Haplorchinae is given. The systematic position of the various species in this group is critically reviewed. The subfamily Haplorchinae and the genus *Haplorchis* are accepted as valid while Monorchotreminae and *Monorchotrema* as synonyms.

2. Three species of *Haplorchis* are found in Kwangtung Province, namely, *H. pumilio* (Looss 1896), *H. taichui* (Nishigori 1924), and *H. yokogawai* (Katsuta 1932).

3. Throughout the work emphasis is laid on the acetabular spines or hooks which are considered to be the only recognisable constant structure practical enough for specific diagnosis.

4. It is believed that there are only four valid species of *Haplorchis*, namely, *H. pumilio*, *H. yokogawai*, *H. taichui*, and *H. milvi*. The validity of *H. milvi*, however, is in question. *H. cahirinus* and *H. anguillarum* do not fit into the diagnosis of the subfamily *Haplorchinae*, while *H. microrchia* and *H. taihokui* fall into synonymy with *H. taichui* and *H. pumilio* respectively.

5. A key for the identification of the known species is given.

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THE PROCESS OF ENCYSTMENT OF A CERCARIA PARASITIC IN *LYMNAEA TENERA EUPHRATICA*

By MIRIAM ROTHSCHILD

(With 8 Figures in the Text)

In 1933 Mr C. Machattie, F.R.C.V.S., D.V.S.M., very kindly sent me a small portion of a specimen of *Lymnaea tenera euphratica* Musson, collected in Iraq, infected with a cercaria belonging to the heterogeneous Gymnocephalic group. Sections were cut and it was found that most of the cercariae had been fixed during various stages of encystment. An excellent opportunity was thus afforded for observing this process, particularly the initial stages.

A brief description of the parthenita and cercaria is given below, but no name has been applied to this apparently new form, for it is doubtful whether a sufficiently detailed description can be made from preserved material.

Technique

The material was fixed in Bouin-Dubosq, and the sections, cut at 6μ, were stained with Delafield's haematoxylin and counterstained with eosin.

Description

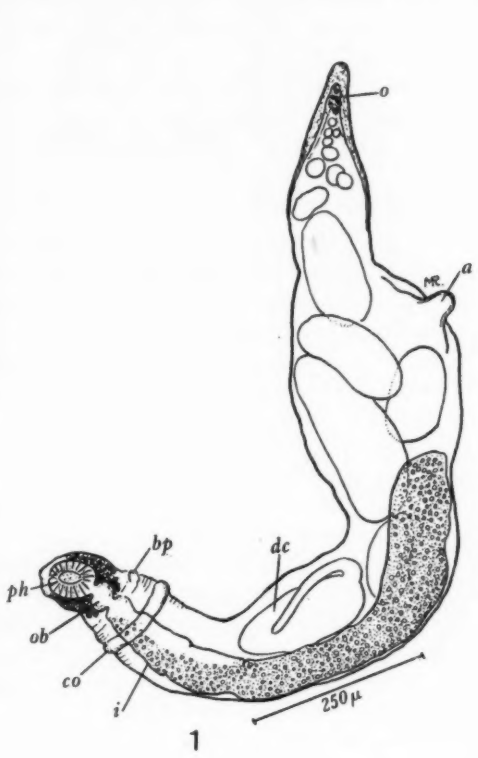
Measurements of redia in μ: maximum length of body, 1600; maximum width of body, 320; diameter of pharynx, 64; length of intestine, 1000; width of intestine, 112.

The redia (Fig. 1) is cylindrical in shape, tapering posteriorly. There is a well-developed pharynx followed by a dilatation, the oesophageal bulb, and an intestine, about one-third the width of the body cavity, reaching to within a third of the posterior extremity. The collar, birth-pore and ambulacral processes are well developed. A few nerve fibres can be seen in the region of the oesophageal bulb. The anterior end is solid, being filled with parenchymatous cells. The ovary is situated at the extreme posterior end of the body. There appear to be between four and five more or less fully developed cercariae in each redia, together with younger stages and germ balls.

The rediae are concentrated in the interlobular spaces of the liver, which is almost entirely destroyed. Occasionally they are present in other organs.

Measurements of maximum-sized cercaria in μ: body length, 256; body width, 160; tail length, 300; tail width without fins, 32; fin width, 30; oral sucker, 42×42; ventral sucker, 32×32; pharynx, 34 long $\times$ 27; oesophagus, 51 long $\times$ 10; intestinal caeca length, 160; intestinal caeca width, 17; distance of ventral sucker from anterior end, 128; cyst diameter, 160; cyst wall, 16.

The cercaria (Figs. 2–5, 7 and 8). The body is oval and broadly rounded at either end. The stout muscular tail is terminal and slightly longer than the body, carrying a fin-fold along the lateral borders. The cuticle is unarmed. Scattered pigment granules are embedded in the outermost layer of the cuticle on the dorsal surface in fully developed cercariae. Both oral and ventral



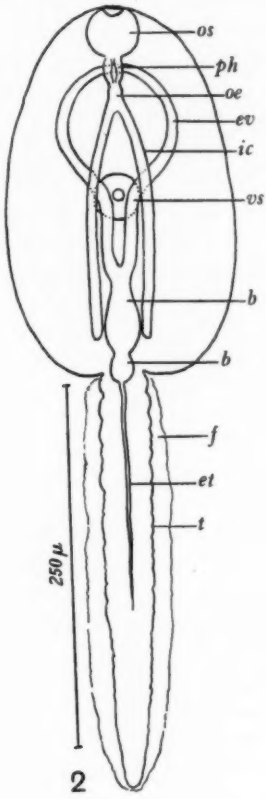


Fig. 1. Redia (whole mount).
Fig. 2. Diagram of full grown cercaria showing main excretory system and alimentary canal.

suckers are well developed, the latter situated a little in front of the middle of the body. The former, which is terminal, leads into a pharynx, followed by a short oesophagus which bifurcates into two large intestinal caeca reaching to the posterior end of the body.

The main excretory system consists of a bladder, shaped like an hour-glass, situated posteriorly (the most posterior dilatation seems to project into the anterior portion of the tail). Two parallel tubes leave the bladder at the anterior extremity of the anterior half, diverge to form a lyre-shaped figure,

and fuse in the region of the oesophagus, thus forming the excretory vesicle. The course of the collecting tubes was not observed. An excretory canal appears to run down the centre of the tail. Its course is marked by falciform cells, the protoplasm of which stains markedly with haematoxylin.

The reproductive organs are poorly developed; they consist of a small group of cells posterior to the ventral sucker and are connected by a short double chain of cells with a somewhat similar group anterior to the ventral sucker.

A central nervous mass lies behind the oesophagus from which anterior and posterior nerve fibres can be traced.

The specialised secretory or gland cells form the most striking feature of the cercaria.

(1) *Cystogenous cells*

(*a*) *Giant ventral gland cells* (Fig. 3). These cells, which number between sixty and eighty, are closely packed and occupy the entire ventral surface of the body excepting the region of the suckers. They measure up to 52 μ along their long axis, which is generally transverse, but the whole cell is often roughly equilateral. The nucleus is very large (13 μ in diameter) and exceedingly poor in chromatin. Its outline is somewhat irregular, but the nuclear membrane is distinct. Small aggregations of chromatin are present.

The cells are loosely filled with large spherical granules, primrose yellow in colour. Their size and shape are rather variable, the largest measuring 5·1 μ in diameter. Occasionally a few of these granules stain red while still within the cell, but more frequently after being shed during encystment.

(*b*) *Cells in the region of the ventral sucker* (Figs. 3–5). These cells number between sixteen and eighteen and surround the ventral sucker except laterally. They are smaller than the ventral cells, only measuring up to 25 μ along the long axis. The nucleus (7 μ in diameter) is more compact and stains deeply, so that the nucleolus cannot always be distinguished. The cells are closely packed with small crystal-like granules of varying shape, not longer than 3 μ. They are pointed at both ends, often spindle-shaped, and stain bright vermilion red, giving the whole cell this characteristic colour.

(2) *"Salivary" gland cells* (Figs. 3–4)

Posterior to the pharynx and immediately anterior to the cells surrounding the ventral sucker are six large cells (35 μ in diameter) which stain an even, carnation pink. The colour and manner of staining is the same as that of the penetration glands of other groups of cercariae. Their function cannot be ascertained with the material available. The nuclei (8 μ in diameter) are irregular in outline and very rich in chromatin, staining deeply throughout. The ducts of these glands are extremely difficult to make out in the sections, but they seem to pass on either side of the pharynx and open on the anterior sucker. They have not been traced throughout their length.

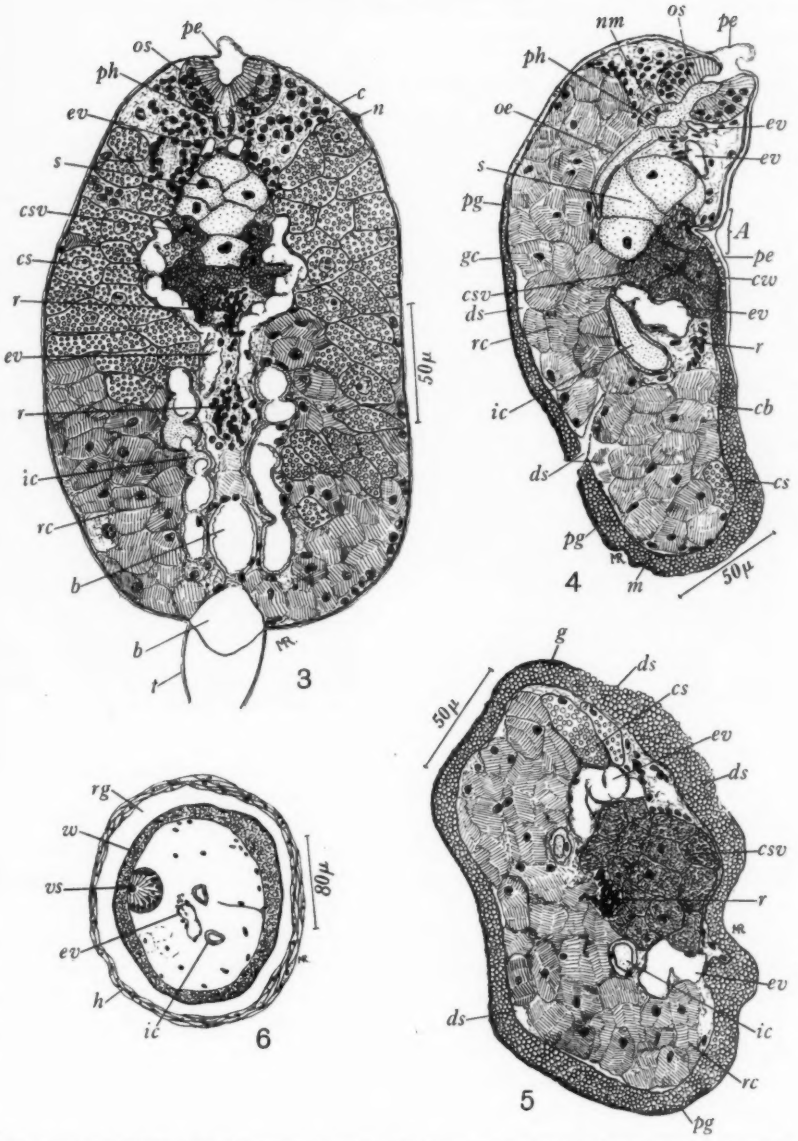


Fig. 3. Longitudinal, slightly diagonal, section of full grown cercaria, showing various types of secretory cells.

Fig. 4. Longitudinal, slightly diagonal, section of cercaria, showing first phase of encystment.

Fig. 5. Transverse section (immediately anterior to the ventral sucker) of encysting cercaria before the cystogenous glands surrounding the ventral sucker have shed their contents.

Fig. 6. Fully formed cyst (rod cells omitted). For explanation of lettering see p. 62.

(3) *Bâtonnet or rod cells* (Figs. 3–5, 7 and 8)

The entire dorsal surface and the greater part of the body is occupied by rather variably sized parenchymatous cells staining light mauve, the largest of which measure about 20μ in diameter. These are entirely filled with "bâtonnets" or "Stäbchenkörper", rod-like inclusions, also staining light mauve, which are arranged symmetrically in groups within the cells. These in no way resemble the granules of the cystogenous cells. The nucleus (8μ in diameter) is irregular in shape and stains rather deeply.

The situation of the ventral cystogenous glands and parenchymatous cells is somewhat similar to that described by Rees (1932) for the cercaria of *Fasciola hepatica* L.

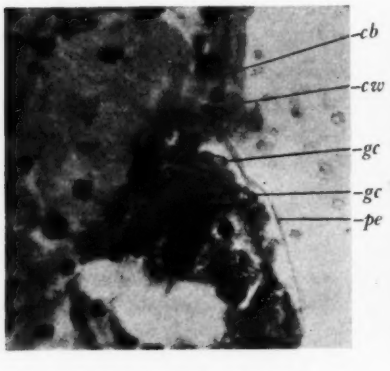
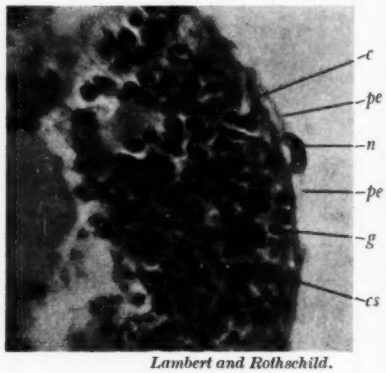


Fig. 7. Photomicrograph of region *A* of Fig. 4, showing cystogenous granules in the cuticle.



Lambert and Rothschild.

Fig. 8. Photomicrograph of cuticle of full grown cercaria showing nucleus of "primitive epithelium".

Site of the cyst. The cercariae were found encysting within the rediae. Isolated cysts were also found in the solid tissue of the snail into which the cercariae must have penetrated.

In nature the cercariae probably encyst in the first intermediate host or in another snail of the same species. It is possible that they may even encyst in the open.

Encystment and the appearance of the cyst (Figs. 4–7). The initial stage in the formation of the cyst is the emptying of the granular contents of the posteriorly-situated giant ventral cells. The cell wall ruptures and a few granules pass out through the muscle layer and basal layer of the cuticle into the outer layer of the cuticle. This outer layer, which stains pale violet in contrast to the more reddish staining basal layer and muscle fibres, now presents a somewhat swollen vacuolated appearance measuring 8μ in depth, instead of the normal 2·5μ (Figs. 4 and 7). As the pressure is increased by the addition of more cystogenous material the granules within the cuticle are

forced round the posterior end of the cercaria and along the dorsal surface (Fig. 4). A further increase in the pressure causes the rupture of the outer layer of the cuticle. The course of the granules is now limited by the larval "primitive epithelium" (Fig. 8) (Dubois, 1929,) which gradually bulges outwards as the granular discharge accumulates beneath it. Meanwhile the rest of the giant ventral gland cells shed their contents which flow along the ventral surface to the region of the oral sucker. At this stage the shape of the cercaria is still distinctly oval and the suckers protrude sharply from the cyst wall which is in process of formation. A grip with the suckers, particularly the ventral sucker, no doubt enables the cercaria to perform a rapid rotating movement. The tail is not yet detached from the body and the sections clearly show that during this time it is lashed from side to side.

As the cyst wall becomes deeper the shape alters, the "cyst" assumes a less elongated outline, and the tail is cast off. It is not until this stage is reached and the giant ventral cells are voided, that the cystogenous cells of the second type, surrounding the ventral sucker, shed their contents.

It is difficult to suggest the precise function of these somewhat elaborately specialised granules. Perhaps they merely serve to strengthen the cyst wall in the region of the ventral sucker where it is decidedly weak. Gradually the cercaria becomes folded upon itself and the cyst assumes a circular outline. At first the penetration and salivary glands can be clearly seen, but in older cysts these disappear and the various other organs become less clearly differentiated. The rod cells, containing the rods or "bâtonnets", are however still visible in the oldest cyst found.

It is impossible to ascertain the age of the oldest cysts, but the condition of the host tissue surrounding them indicates that they must have been present within the snail for a certain time. The cyst wall consists of

(1) An extremely thin, faintly pigmented outer covering, formed by the primitive epithelium and the pigment granules from the outer layer of the cuticle of the cercaria which are carried away by the cystogenous granules when they rupture the cuticle.

(2) A layer 16μ deep, which at this stage has practically solidified into a homogeneous mass, although its granular origin is still evident. Between the cyst wall and the tissues of the snail there appears to be a space of about 16–32μ, perhaps filled with fluid from the host, while surrounding this are several layers of thickened tissue of the snail, which consist of tough, elongated, spindle-shaped cells (Fig. 6).

Numerous cysts of another very small distome were found in the mantle and other organs of the snail. The cyst wall of these parasites is very different, but the reaction of the host's tissue is similar though less pronounced, the surrounding capsule being only one or two layers deep.

The most interesting feature in the encystment of the cercaria is the unusual mechanism involved which ensures that the granular cystogenous material follows the contours of the body. The retention of the larval primitive

epithelium until so late a stage in the development of the cercaria is exceptional. In most types of cyst formation the pouring forth of the granular matter is preceded by the exudation of a hyaline mass or flow of mucus which, on solidifying, forms the thin outermost layer of the cyst wall. Wunder (1924, 1932) and Bovien (1931), however, are of the opinion that in certain species studied by them the "cuticula" of the cercaria swells up and that the cystogenous material is deposited within it.

The nature and function of the Stäbchenkörper or rod cells present in the parenchymatous cells require further investigation. Leuckart (1882) supposed that they were myoblastic and gave rise to muscle fibres. Wunder (1932) observed that apparently similar structures (in *Cercaria tuberculata* Fil., *C. coronata* Fil., *C. limbifera* Seif., *C. monostomi* v. Linst.) migrated from the cells of their origin and broke down into the granular and semi-liquid cystogenous material which eventually formed the cyst wall. In the cercaria described here this process does not appear to take place, for not only are the rod-like inclusions still present in the parenchymatous cells after the cysts have been formed for some time, but much of the granular contents of the cystogenous glands is formed before the bâtonnets are fully developed. It is possible that the rods originally played some part in cyst formation, a function which is now lost in this and other allied species of cercariae.

ACKNOWLEDGMENTS

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Explanation of Lettering

a, ambulacral process; *b*, bladder; *bp*, birth pore; *c*, cuticle; *cb*, basal layer of cuticle; *co*, collar; *cs*, cystogenous gland cells; *cw*, swollen cuticle; *csv*, cystogenous gland cells surrounding ventral sucker; *dc*, outline of developing cercaria; *ds*, damaged portion of section; *et*, caudal excretory tube; *ev*, excretory vesicle; *f*, fin fold; *g*, cystogenous granules; *gc*, cystogenous granules within the cuticle; *h*, capsule formed by host; *i*, intestine; *ic*, intestinal caeca; *m*, muscle layer; *n*, nucleus in primitive epithelium; *nm*, central nervous mass; *o*, ovary; *ob*, oesophageal bulb; *oe*, oesophagus; *os*, oral sucker; *pe*, primitive epithelium; *pg*, pigment granules; *ph*, pharynx; *r*, developing reproductive organs; *rc*, rod cells; *rg*, region between cyst and host tissue; *s*, salivary gland cells; *t*, tail; *vs*, ventral sucker; *w*, cyst wall.

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NEW PARASITIC COPEPODA FROM NAPLES

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(With 9 Figures in the Text)

This collection, among others, was made for and kindly presented to me by Miss Miriam Rothschild during her visit to the Zoological Station, Naples, in April 1934.

The type specimens mounted in euparal are deposited in the British Museum.

DICHELESTHIIDAE

Hatschekia ischnon n.sp.

Host. *Pagellus erythrinus* Cuvier.
Location. Gills.
Date. 8. iv. 1934.
Material. 1 ♀ (Figs. 1 and 3).

Diagnosis. *Cephalon* nearly spherical, a little wider than long. *Neck* very short and only indistinctly segmented. *Trunk* narrow, elongated, wider anteriorly than posteriorly (as in *Hatschekia gerro*, but an unusual feature), gradually tapering, more than twice as long as wide, the postero-lateral corners produced into rounded lobes not at the side of the abdomen. *Abdomen* spherical, prominent, with uniarticulate appendages each bearing two setae.

Appendages. *Antennules* three-articled with four fine setae on the distal article, two apical and two lateral. *Antennae* shorter and stouter than in *H. gerro*, two-articled; the proximal article is an enlarged base with a large strong hook shutting down on to it.

Maxillipedes consist of two long articles, and a small distal article, the proximal article elongated on which shuts down the distal article with two unequal apical setae.

Pereiopods two pairs, biramose, each ramus biarticulate with two apical setae. Basal portion elongated, two-articled.

Hatschekia gerro n.sp.

Host. *Labrus turdus*. Bl.
Location. Gills.
Date. 8. iv. 1934.
Material. 2 ♀♀ (Figs. 2 and 4).

Diagnosis. *Cephalon* much broader than long, with slight postero-lateral lobes. *Neck* stout, indistinctly segmented, somewhat lobed laterally. *Trunk*

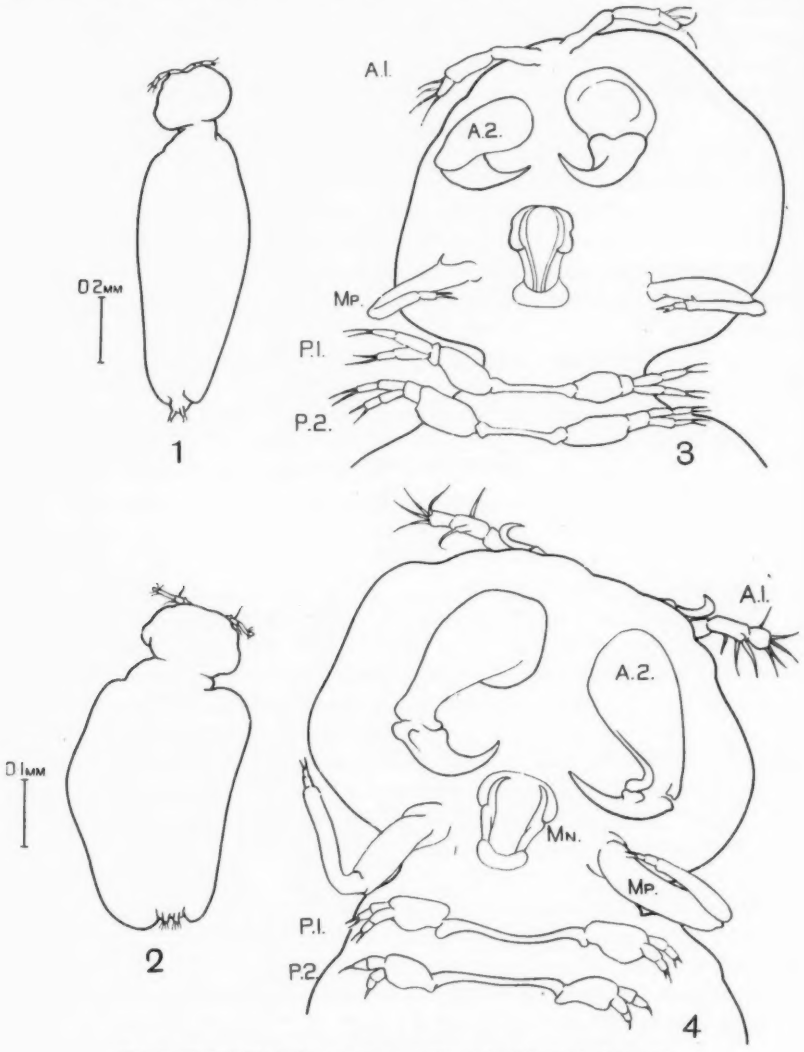

Fig. 1. *Hatschekia ischnon* ♀, n.sp., entire animal in ventral aspect.
Fig. 2. *Hatschekia gerro* ♀, n.sp., entire animal in ventral aspect.
Fig. 3. *Hatschekia ischnon* ♀, n.sp., anterior end in ventral aspect.
Fig. 4. *Hatschekia gerro* ♀, n.sp., anterior end in ventral aspect.

stout, wider anteriorly than posteriorly, width five-sevenths of the length, the postero-lateral corners are produced into rounded lobes which are longer than

and conceal the abdomen. *Abdomen* hardly differentiated, with uniarticulate appendages each bearing four setae.

Appendages. *Antennules* three-articled and setigerous, the basal article with an anteriorly recurved hook. *Antennae* two-articled, very large, the proximal article elongated, the distal article a sharp curved claw. *Mandibles* curved and toothless.

Maxillipedes elongated and slender, resembling those of *Hatschekia hippoglossi* (Krøyer), consisting of two long articles capped by one or indistinctly two small articles with apical setae. Basal article stouter than in *H. hippoglossi*, the other articles at an angle to it.

Pereiopods two pairs as in *H. ischnon* but smaller, not projecting beyond the margin of the neck; base stout with only one article.

LERNAEOPODIDAE

Alella berecynthia n.sp.

Host. *Crenilabrus* sp.

Location. Gills.

Date. 8. iv. 1934.

Material. 1 ♀ (Figs. 5 and 6).

Record. The genus *Alella* Leigh-Sharpe (1925, p. 200) was erected to include those forms resembling *Clavella* but which:

(1) Are not parasitic on Gadoid fishes (one exception).

(2) Have the second maxillae entirely free or fused only at their distal half. (*A. alata* (Brian, 1906)).

(3) The bases of the second maxillae bear on their outer sides, at their junction with the cephalothorax, spherical extensions variously described as horn-like, gibbous or wing-like (the present new species an exception). To these may be added:

(4) The antennule has an exopodite.

(5) The bulla is aberrant; chitinous bristles in a fascicle (*A. canthari* (Heller 1865)); a spherical knob (*A. alata*); bilaterally symmetrical as in *Salmincola* (*Alella berecynthia*); but never clavate as in *Clavella*.

Diagnosis. *Body*, small, *Cephalothorax*, not so long as trunk, straight. *Head* not enlarged. Base of *neck* not much enlarged. *Trunk*, narrow, cylindrical, of the same diameter throughout, one and a quarter times as long as wide. *Second maxillae*, nearly as long as cephalothorax and in same straight line with it, entirely free; the *bulla* appears to be attached to only one of the maxillae (probably abnormal), and is bilaterally symmetrical, resembling a boot, more like that of some species of *Salmincola* than of any other genus, without channels; no discoid terminations to the maxillae. *Abdomen* spherical, very small without appendages. *Ovisacs*, one and half times as long as the trunk, and together equalling it in breadth. *Eggs* large and few, only two rows apparent in lateral aspect, about twelve in a row.

Appendages (Fig. 6):

Antennules four-articled, the basal article much enlarged with a spiral turn upon itself. Of the other three articles the middle is the smallest. The distal article bears five mammillations one of which is larger than the rest and is provided with a spine.

Antennae consist of a large stout basal portion bearing a large unjointed endopodite provided with several mammillated spines, a bulging prominence on the outer border, and subapically a pad of spines, and a prominent exopodite whose diameter is equal to that of the endopodite as regards the enlarged basal article, the terminal article of the two bearing one prominent spine apically, and one subapical spine.

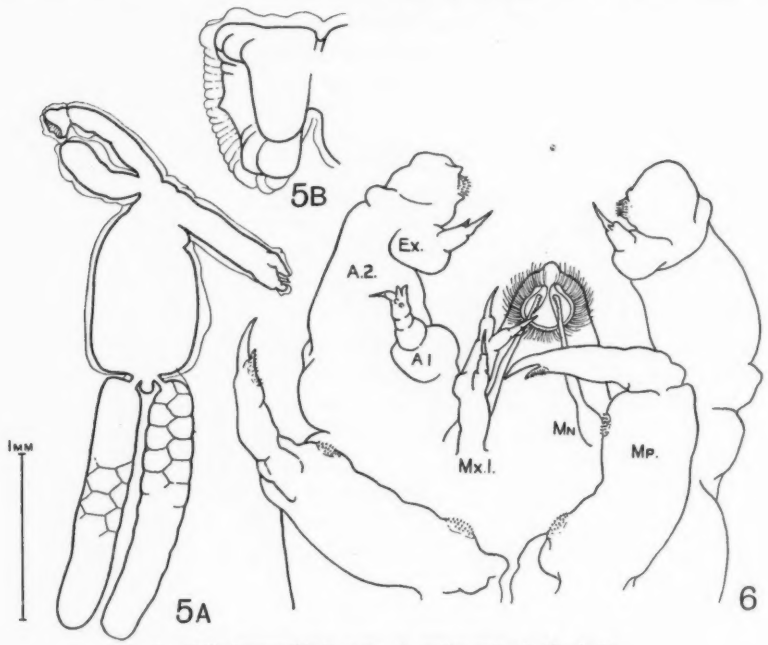

Fig. 5. *Alella berecynthia* ♀, n.sp., A, entire animal. B = bulla.
Fig. 6. *Alella berecynthia* ♀, n.sp., anterior end in ventral aspect.

Mouth cone prominent, with well marked setae.

Mandibles slender, acicular, with seven prominent teeth.

First maxillae with an unsegmented endopodite, bipartite at the distal end, one ramus being terminal and the other on the inner margin; each ramus ends in a long soft spine; and an expodite resembling one of the above rami.

Second maxillae described above.

Maxillipedes two-articled, the basal long and slender provided with two rounded cushions covered with spines, the proximal nearer the body than is

usual. There is no spine nor hook between the cushions. Terminal article long and slender, ending in a powerful slightly-curved claw with an accessory claw at its base set immediately above a cushion of spines.

Whereas the first maxillae resemble those of *Clavella*, the other appendages, especially the two antennae and maxillipedes, are more like those of *Lernaeopoda*.

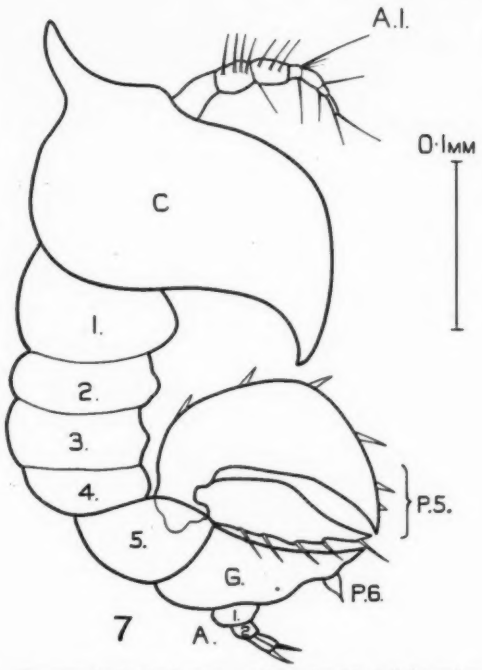

Fig. 7. *Parategastes haphe* ♀, n.sp., entire animal in left lateral aspect.

TEGASTIDAE

Parategastes haphe n.sp.

Host. *Serranus hepatus* L.

Location. Gills.

Date. 10. iv. 1934.

Material. 1 ♀ (Fig. 7).

Diagnosis. *Body* curled forward both anteriorly and posteriorly, a dense, opaque black. *Cephalothorax* concealed, with the lateral portions produced both dorsally and ventrally into large laminae ending in a large horn.

First thoracic segment not fused with the cephalon.

Fifth segment not larger than the first. *Sixth or genital segment* elongated terminating in a spine, longer than wide. *Abdomen* of two segments, each

somewhat spherical, the anterior the larger. *Abdominal appendages* longer than wide, each capped by a long seta.

Appendages: *Antennules* seven-articled, setose, the articles unequal in size, curved forward.

Fifth pereiopods biramous, each ramus uniarticulate. Endopodite elongated, a flat, falciform plate ending in a point, with six unequal marginal spines on the convex border. Exopodite elongated, a flat plate ending in a blunt spine, with five lateral setae on its outer margin. These podites are modified in relation to the parasitic habit, acting like blunt scissor-blades, a portion of the gill of the host being clipped between their concave edges.

Sixth pereiopods mammillated, ending in a spine, and much larger than in other species.

Other pereiopods present but cannot be distinguished.

ISMARDIDAE

As I am unable to locate the new Harpacticoid genus *Ismardis* in any known family it is placed for the present in a new family Ismardidae having the characters of the new genus, and showing a superficial resemblance to the Thalestridae, especially *Pseudothalestris*, but not in the appendages.

Ismardis n.gen.

Ismardis spartacus n.sp.

Host. *Patella coerulea* Lam.

Location. Gonad and mantle cavity.

Date. 8. iv. 1934.

Material. 2♀♀, 1♂ (Figs. 8 and 9). Colour—scarlet (in alcohol).

Diagnosis. *Body* short, about 0·1 mm. in length, stout, pear-shaped and arched dorsally; sexual dimorphism very marked; *cephalon* large, fused with the first two thoracic segments; urosome sharply defined from the metasome; *abdomen* of four segments tapering posteriorly; *abdominal appendages* slender, longer than wide, apical seta very elongated, one or two small lateral setae.

Rostrum bluntly rounded in the female, but in the male very pronounced and curved forward ventrally, forming a hook. *Genital segment* not markedly divided *T* (6+7).

Appendages. *Antennules* (A 1) seven-articled. In the female two large proximal and five small distal articles; all are fringed with elongated setae on the outer border and a few small setae at the joints on the inner side. In the male the appendage is modified to form a clasping organ armed with a few elongated setae, the proximal article with numerous small stiff curved spines, the third article with a mammillated spine on the outer edge; the terminal portion composed of two segments, the sixth article spatulate.

Antennae. Biramous in the male, uniramous in the female. Basal article

more slender in the male, the rami uniarticulate with elongated apical setae; two lateral setae in the female.

Second maxillae two-articled, the distal article capped by four setae and a mammillated spine.

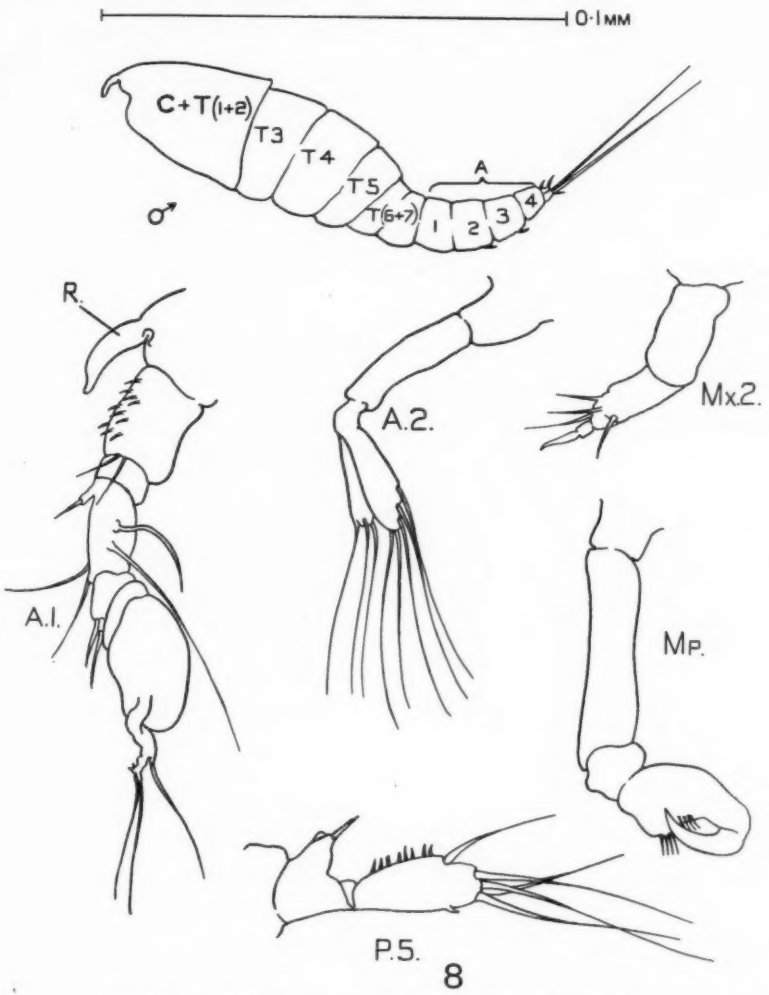

Fig. 8. *Ismardis spartacus* ♂, n.sp., lateral outline and appendages.

Maxillipedes four-articled, the distal article a powerful claw which flexes against the third where it is setose. Proximal article longer and slenderer in the male. Third article longer and stouter in the female.

Pereiopods uniarticulate and biramous:

1*st pair.* Each ramus biarticulate; distal article with large setae, and larger on the exopodite. Proximal article of the endopodite with small setae.

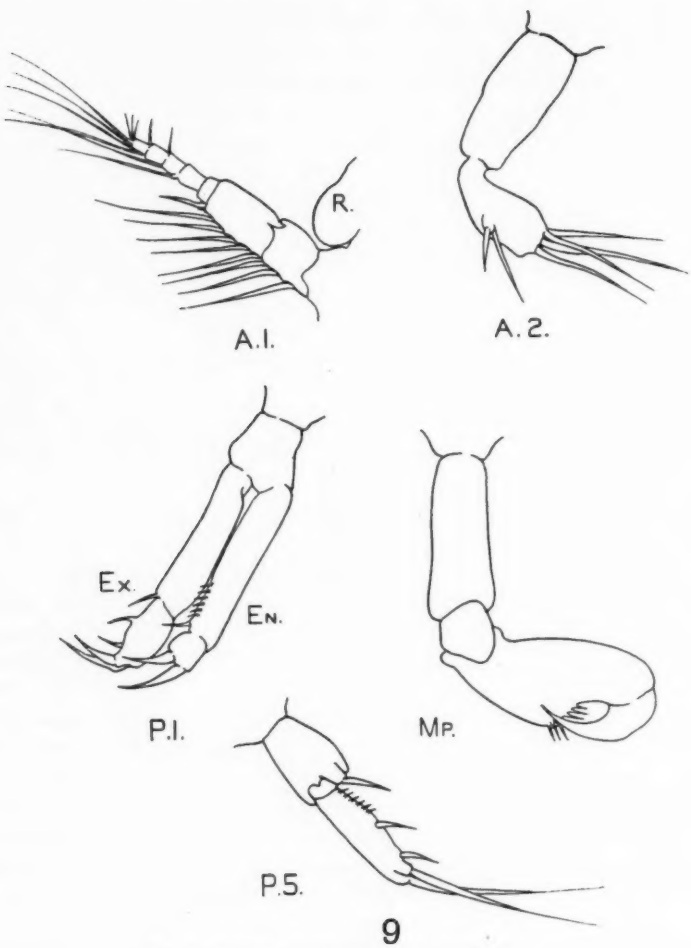

Fig. 9. *Ismardis spartacus* ♀, n.sp., appendages.

5th Pair. Uniramous; the basal portion with a lateral projection bearing a spine, more pronounced in the male; the ramus ending in four elongated apical and one subapical setae and a few small lateral ones, in the female two long apical, two short lateral and numerous small lateral setae. The ramus is more slender in the female.

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Explanation of the lettering of figs. 1–9.

A. = abdomen; *A.* 1 = antennules; *A.* 2 = antennae; *B.* = bulla; *C.* = cephalothorax; *En.* = endopodite; *Ex.* = exopodite; *G.* = genital segment; *Mn.* = mandible; *Mp.* = maxillipede; *Mx.* 1 = first maxilla; *Mx.* 2 = second maxilla; *P.* 1–6 = pereiopods; *R.* = rostrum; *T.* = thorax.

(*MS. received for publication* 30. v. 1935.—Ed.)

CHLOROMYXUM PRISTIOPHORI, A NEW SPECIES OF MYXOSPORIDIA PARASITIC IN THE GALL-BLADDER OF *PRISTIOPHORUS CIRRATUS* (SAW-SHARK)

By VIOLET WOOLCOCK

From the Department of Zoology, University of Melbourne

(With Plates I and II, containing Figs. 1–19)

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Introduction

Within recent years a considerable amount of work has been done on the Myxosporidia, especially, as pointed out by Kudo (1919), in North America, Asia, and to a lesser degree in Japan (Fugita, 1912, 1913, 1925). Few investigations, however, have been carried out in Australia, and consequently very little is known concerning the distribution of the group in this country. There appear to be as yet only two contributions to the subject, papers by T. Harvey Johnston and M. J. Bancroft (1918, 1919). As far as can be ascertained, my paper is the first report concerning Myxosporidia from fishes of Southern Australian waters, and contains the first record of a species of *Chloromyxum* from Australia.

The parasite inhabits the gall-bladder of the saw-shark *Pristiophorus cirratus*, and every fish examined was found to be infested, the infestation being usually very heavy.

It is interesting to note that an investigation of a large number of the common gummy-shark, *Mustelus antarcticus*, and the Port Jackson shark, *Heterodontus philippi*, failed to reveal any trace of a Sporozoan infestation.

Material and methods

The investigation, on which the following description is based, was carried out on material collected from Port Philip Bay, Victoria, at all times of the year. This included an examination of living material, smears of the bile, intestinal smears, and sections of the gall-bladder and bile duct. For the

smears, fixatives such as Bouin's, Zenker's, Carnoy's and Schaudinn's were used. The latter was found to be most successful, while Giemsa's staining, although not very consistent, gave the best results for nuclear stages. Delafield's haematoxylin stained later developing spore stages well, while iron haematoxylin, though proving rather unsatisfactory for smears, was good for sections of the gall-bladder and bile duct. For the spores, the method recommended by Davis (1915) was used; the measurements given, however, are from the fresh material.

A definition of certain terms employed is given below:

Pansporoblast: the enclosed area in the endoplasm of the vegetative form, in which two sporoblasts become differentiated.

Length of spore: the sutural diameter.

Breadth: the largest diameter of the spore measured at right angles to the length or sutural diameter, the smaller diameter being the thickness.

Front view: the view in which the length and breadth of the spore are laid horizontally.

In addition the terms capsular and post-capsular sides or ends as proposed by Davis (1915), in place of anterior and posterior ends, are employed here.

MORPHOLOGY AND CYTOLOGY OF *CHLOROMYXUM PRISTIOPHORI*

(*a*) *Vegetative form*

The plasmodia, which consist of amoeboid protoplasmic masses ranging in size from 10 to 600μ, are in form extremely polymorphic, their shape varying from broad massive to circular, oval, or narrow elongate. The pseudopodia, arising only from the anterior end, are sometimes short and pointed, but may change to a lobose condition in one and the same individual (Figs. 1 and 2). In the formation of the lobose type of pseudopodia (Fig. 3) the billowing movement of the ectoplasm is extremely characteristic. Old spore-bearing individuals (Fig. 4) have no pseudopodia.

In life the ectoplasm varies in colour from clear hyaline to a rather dark green. It is not always clearly differentiated in the resting state, and no pseudopodia are then visible. A pellicle is definite except at the place of formation of pseudopodia.

In the endoplasm of the living animal the green refractile granules, characteristic of *Chloromyxum* species, are to be observed. Like the ectoplasm the colour of the endoplasm varies from almost colourless to darkish green, and this may be intensified by the presence of numerous granules which vary both in size and shape.

Some small spherical granules are yellowish, and presumably correspond to the "colour-carriers" of Erdmann (1916), while others, also more or less spherical and of varying sizes, are green, and are possibly identical with her "reserve" bodies. In addition to these two types there are to be seen large spindle-shaped granules, often but not always associated with the former.

In stained specimens the three types of nuclei usually present in young Myxosporidian plasmodia are noticeable. These are firstly, nuclei with a large central karyosome and a chromatin thread on the nuclear membrane (Fig. 11*a*). These are presumably the vegetative nuclei, and they are to be found scattered throughout the plasmodium; a cell membrane is never apparent.

To the second type belong the nuclei of the so-called "propagative cells of the first order" of Keysselitz (1908) (megalocytes of Dunkerly (1925), and isogametocytes of Erdmann (1916)). Smaller in size, and with less conspicuous nucleoli, these nuclei are at once distinguished from the vegetative nuclei by the appearance of the surrounding plasma. This is rather denser in structure than the remaining endoplasm and consequently stains more deeply. In addition the cell membrane is here always well defined, while the cytoplasm itself usually presents a finely alveolar construction. With Giemsa's stain, however, sometimes a narrow concentrated granular zone immediately surrounding the nucleus is revealed (Fig. 10 *b*).

The nuclei of the third type are likewise smaller than the vegetative nuclei, and are to be distinguished from the second type by the more compact nature of their chromatin (Fig. 10 *c*). They are similarly nuclei of generative cells, the "propagative cells of the second order" of Keysselitz (microcytes of Dunkerly), and are to be found associated with the second type of nuclei clustered together in varying numbers in a pansporoblast. The cytoplasm offers no distinctive features, and the cell membrane is often but not always visible. On account of the variability of this latter feature, stages succeeding the four-cell stage are referred to as "multi-nucleate" rather than "multicellular".

Mature trophozoites may have, in addition, spores in various stages of development. Of these, polysporous forms (Fig. 4) are prevalent, the disporous and monosporous types (Fig. 5) being only rarely seen. There is no evidence, however, whether these two latter are in themselves complete individuals or merely fragments of a disrupted polysporous form.

(*b*) *The spore*

The spores (Fig. 6*a–e*) are ovoidal, measuring 11 μ in length by 8–9 μ. The four polar capsules lying together at one end are large, of equal size, and measure 5–6 μ in length, by a maximum breadth of slightly less than 3 μ towards the base. Each spore valve is marked by five ridges which run parallel to the sutural line. The ridges are invisible shortly after passing the bases of the polar capsules, becoming fainter towards the capsular end. The serrated edge (Fig. 6*a*) to which they give rise, is here quite definite even in fresh spores.

As with *C. leydigi*, which this species most closely resembles, the sutural line is narrow but distinct, and filaments grow out from the truncated anti-capsular end of the spore as in *C. leydigi*.

Mature spores, that is to say spores in which the only nuclei present are those of the sporoplasm, were not found in the bile. The capsular nuclei were

always present as well, although often in process of disappearing. This criterion of a mature spore, however, may not be general, and certainly Auerbach (1909) illustrates ripe spores of *Myxidium sphaericum* and *Sphaeromyxa hellandi*, with a nucleus at the base of each of the polar capsules.

The spores were occasionally found free in the bile, but more often were surrounded by a clear, hyaline, more or less hemispherical membranous envelope (Fig. 7 *a–d*), in which, in the fresh material, some of the refractile green granules referred to previously were to be seen. The significance of these envelopes is discussed later.

Methods recommended for bringing about extrusion of polar filaments were tried, but without success. In a number of capsules it was possible, with appropriate staining, to count five well-separated coils of the thread.

METHODS OF PROPAGATION

(*a*) *Asexual*

Multiplication by plasmotomy and exogenous budding were occasionally seen (Fig. 8 *a*, *b*), while forms with endogenous buds were fairly common. The latter were clearly distinguishable in the living trophozoites by their lighter appearance, while in stained specimens each bud was surrounded by an almost colourless demarcating rim. The buds, which were usually rounded, measured from 20 to 45 μ in diameter, while the number found in a plasmodium varied from one to six (Figs. 9–12).

An examination of sections of the gall-bladder failed to reveal any schizont formation in the epithelial cells as described by some authors.

(*b*) *Sexual: spore formation*

In regard to the formation of the spore-anlagen, the species here described does not appear to differ from other species of *Chloromyxum*, although in later stages a slight variation is apparent.

As in *C. leydigi* the two-cell stage of the pansporoblast is formed by the association of two propagative cells of the first order (Fig. 13*a*). Intermediate stages between the propagative and the vegetative nuclei are to be observed, and it is presumed therefore that here also they are derived from the vegetative nuclei as stated by Erdmann (1916) for the type species. Although mitoses are only very rarely seen, such as are present indicate that propagative cells of the second order arise by heteropole division of the first order cells. Fig. 13*b* shows the transition of a two-cell to a three-cell stage, while Fig. 13 *c* shows the transition of a three-cell to a four-cell stage, and the completion of the spore-anlage. From these two latter figures it can be seen that the division of the large cells does not take place synchronously, this being, as pointed out by Dunkerly, favourable evidence for their separate origin. In the four-cell stage there was no evidence of any nuclear or cytoplasmic fusion as is described

by some authors for related species. Figs. 13 *d–h* and 14 *a–b* present different views of the four-cell stage, some of which are similar to stages illustrated by Erdmann (1916, Pl. 19, fig. 34, and Pl. 20, figs. 51, 52) for *C. leydigi*. The cells of these groups of four, the spore-anlagen, are at first unequal in size, but the smaller appear to grow before division.

The six-nucleate stage (cell membranes being no longer always visible) arises by the division of second-order propagative cells, and both here and in later divisions the recent products of fission are always more deeply staining, later becoming indistinguishable from the other generative nuclei.

Since after the six-nucleate stage the two first-order propagative cells are no longer to be distinguished, it is not possible to say whether or not they take any part in the multiplication of the nuclei of later stages. Fig. 14 *c* shows a six-nucleate stage in which some chromatin is being extruded from two nuclei. These nuclei are possibly the envelope or "resting" nuclei of Schröder (1910), which, however, are non-functional here also, since there is no primary sporoblast envelope formed as in *Myxobolus pfeifferi* (Keysselitz, 1908); and the emission of chromatin from the nuclei may presumably be regarded as a "reduction" process (distinct from the reduction process in which the chromatin is halved), a preliminary stage to their disappearance.

The pansporoblast increases in size, and the nuclei which have multiplied up to twenty (Fig. 17) are still undifferentiated. This stage is comparable to the "syncytium" stage of Mercier (1909). Intermediate stages are shown in Fig. 14 *d* to Fig. 16.

At the twenty-nucleate stage the pansporoblast, which is a dispore sporoblast, becomes divided into two halves. The nuclei arrange themselves in groups of nine, two of the twenty nuclei not taking part in the processes which follow. The differentiation of nuclei of each group now commences. Two become surrounded by a clearly defined cell membrane, and these nuclei can be followed through, later developing spore stages as the nuclei of the sporoplasm. Four other nuclei initiate the formation of the polar capsules. Erdmann considers that a part of each nucleus becomes constricted off to form the capsule. There is no evidence of this here. For some time the nuclei appear to retain their normal size and shape (Fig. 19 *a*, *b*), and only when the capsules appear fully formed are the nuclei pressed to a crescentic shape (Fig. 19 *c*), when they stain more intensely and present a rather pycnotic appearance. The two nuclei associated with the zone of formation of the spore valves appear larger than the others, but are to be distinguished chiefly by their vesicular appearance.

Eight nuclei of each group are thus accounted for; the ninth is presumably concerned with the formation of the membranous envelope which surrounds each spore. For a short time such nuclei may be seen applied to the outer rim or in the immediate vicinity of the developing spore valves, but later, together with the spore valve nuclei, they disappear. There is evidence that the spores leave the mother organism surrounded by this hyaline envelope.

DISCUSSION

The significance of the formation of a secondary envelope, presumably to be regarded as a secondary sporoblast, is not apparent. It was at first thought that it might have some protective function for the spore in its passage to the exterior. An examination of the sections of the bile duct, however, revealed the presence of numerous envelopes in its lumen, but no spores, the spores having evidently passed on to the intestine. Later examination of intestinal contents also failed to shed any light on the function of the envelopes. No structures which might definitely be taken for spores were to be found even in cases where there were numerous sporulating individuals in the gall-bladder, so that the spores must undoubtedly pass very rapidly to the exterior.

Concerning the systematic position of the form here described, of the known species of *Chloromyxum* it most nearly approaches *C. leydigi*, differing from it, however, in its larger size, in the comparatively larger polar capsules, and in the possession of the secondary sporoblast envelope.

In conclusion I wish to say that the presence of the parasite in the gall-bladder of *Pristiophorus cirratus* was first noted by Dr O. W. Tiegs, Associate-Professor in Zoology, University of Melbourne, to whom I am indebted for some of the material used in the above description.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. Camera lucida drawing of living animal.
Fig. 2. Same animal five minutes later.
Fig. 3. Living animal showing lobose pseudopodia.
Fig. 4. Mature spore-bearing individual. Schaudinn-Delafield preparation.
Fig. 5. Plasmodium with single spore. Schaudinn-Delafield preparation.
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Fig. 9. Plasmodium showing single endogenous bud. Schaudinn-Delafield preparation.

PLATE II

Fig. 10. Plasmodium showing three endogenous buds. *b*, first order propagative cell; *c*, second order propagative cell. Schaudinn-Giemsa preparation.

Fig. 11. Plasmodium with one endogenous bud; *a*, vegetative nucleus. Schaudinn-Giemsa preparation.

Fig. 12. Plasmodium with six endogenous buds. Schaudinn-Delafield preparation.

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Fig. 14. *a–b*, further views of the four-cell stage; *c*, six-nucleate stage, from two of the nuclei some chromatin is being emitted; *d*, pansporoblast showing nine nuclei.

Fig. 15. Portion of a plasmodium showing pansporoblasts containing twelve to fifteen nuclei.

Fig. 16. Portion of a plasmodium showing pansporoblasts with eighteen or nineteen nuclei.

Fig. 17. Single pansporoblast with twenty nuclei.

Fig. 18. Pansporoblast with twenty nuclei showing commencement of grouping to form the two spores.

Fig. 19. *a–c*, later stages in spore formation.

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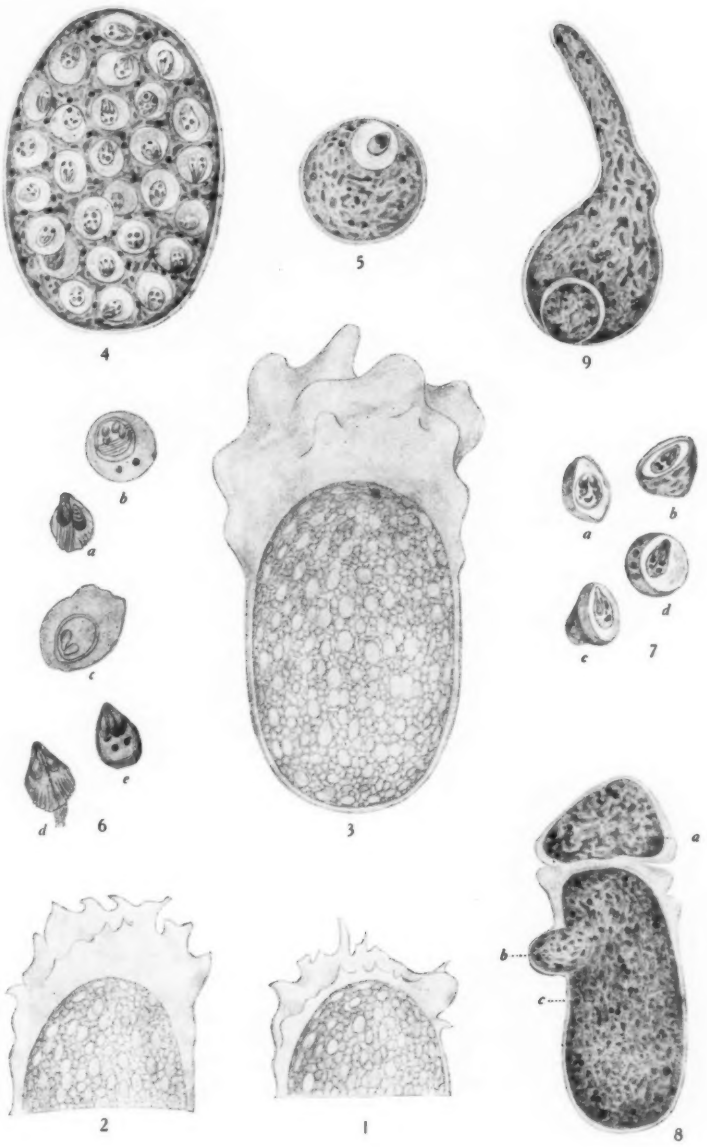
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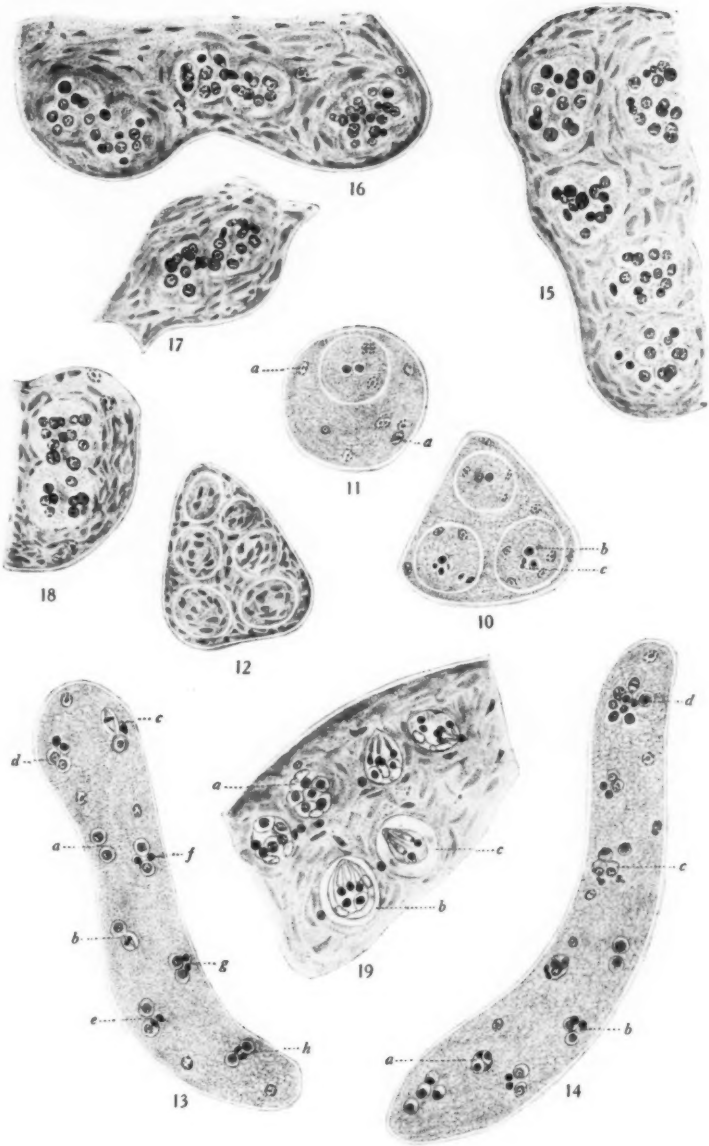
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MONOGENETIC TREMATODES FROM SOME AUSTRALIAN FISHES

By VIOLET WOOLCOCK

From the Department of Zoology, University of Melbourne

(With Plate III and 4 Text-figures)

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Introduction

The small group of Trematodes here discussed comprises new species of the genera *Megalocotyle*, *Calicotyle* and *Microcotyle*.

The new species of *Calicotyle* offers a point of interest in its unusual endoparasitic habitat, being found in pouch-like dilatations of the oviducts of the female of *Pristiophorus cirratus*. In the generic diagnosis, however, provision is made for such a situation, since, it may be recalled, that the type species *Calicotyle kroyeri* was sometimes found not only externally near the anus but also in the rectum.

In determining the systematic position of *Microcotyle victoriae* it became necessary to re-examine *M. bassensis* (Murray, 1931) to obtain some essential details of structure and measurements not included in the original description. These data are briefly recorded here.

In the preparation of mounted specimens the worms were fixed, under pressure of thin glass, in formalin or in Bouin's fixing fluid. Delafield's haematoxylin, iron haematoxylin and alum cochineal were the stains employed. Attempts to reveal the peripheral nerves and nerve cells of *Calicotyle inermis* with methylene blue intra-vitam staining were only partially successful.

Family TRISTOMATIDAE

Megalocotyle helicoleni n.sp. (Pl. III, fig. 1)

Marine form, found on the gills of *Helicolenus percoides* (red gurnet).

Locality. Port Philip Bay, Victoria.

Incidence. The gills of a large number of fish were examined, but only a single specimen of this species was obtained.

Length of formalinised specimen 5·5 mm.; maximum breadth 2·75 mm. Integument smooth, without spines. Body white, translucent in life, oval and flattened dorso-ventrally. Eye-spots, two pairs. Anterior suckers at the anterior extremity of the body, weakly muscular; posterior disc or cotylophore 1·5 mm. in diameter, with marginal membrane thrown into folds. As in the type species, the internal surface of the disc is divided into a central pentagon and seven peripheral areas, by five radiating, slightly raised septa of which the posterior two bifurcate; hindmost area continuous with the central.

Hooks, three pairs, imbedded one behind the other in the two posterior septa, and protruding upon the ventral surface. The most anterior pair (Pl. III, fig. 2), which are considerably larger and stouter than the others, are 0·28 mm. in length. The base of each is only slightly bifurcated, while radiating from the tip to the left side are a number of fine furrows which give to the hook a striated appearance. The middle pair of hooks are simple, slender and slightly twisted, and measure a little less than 0·14 mm. in length. The posterior end of each is provided with a very distinct recurved hook, the anterior end with a small process at right angles to the main axis (Pl. III, fig. 3). The most posterior pair of hooks appear very similar to the middle pair at first sight but differ in that the anterior end of each is divided into two by a deep cleft, one arm so formed having a broad lateral triangular process (Pl. III, fig. 4). The two small hooks of either side lie closely together, being separated from the larger by a distance approximately equal to their own length.

At intervals along the margin between the membrane and body of the disc are to be found a number of small chitinous rods about 9μ in length. It was not possible to obtain an accurate count of the number of these, but from the disposition of those visible it may be presumed that there are fourteen as in the type. The rods, however, are not simple in structure as in *Megalocotyle marginata*. Here there is a small lateral outgrowth about half-way along the length of each, while at the posterior tip are two very minute recurved hooks (Pl. III, fig. 5). The margin of the disc is slightly indented at the point of these rods.

The mouth, situated typically on the ventral surface and in this species slightly posterior to the anterior suckers, opens immediately into a well-developed pharynx, while the intestinal limbs give off numerous diverticula laterally and possibly some medially behind the genital organs. Here, however,

owing to the presence of a very large number of vitelline follicles, the caeca are only faintly visible.

The genital system is situated in the anterior half of the body between the intestinal limbs. In general plan there is a considerable resemblance to that of *M. marginata*; the organs, however, are orientated rather differently in respect to one another. The testes are ovate and median, lying side by side half-way along the body length. The short vasa efferentia arise on the inner border of each testis and almost immediately unite to form the long vas deferens which, owing to the presence of numerous sperms, is easily traceable throughout its course. This vessel is at first directed anteriorly (Pl. III, fig. 1), passing on the left of the ovary. At the level of the yolk reservoir which lies dorsal to it, the vas deferens turns to the right, now passing in front of the ovary. Here it folds back on itself, and on nearing the receptaculum seminis, where it is again directed forwards for a short distance, it becomes rather wider and slightly coiled to form a vesicula seminalis. A second right-hand turn now follows, the vessel passing transversely and then curving over to enter into the prostatic gland, a large more or less ovoid body situated to the right of the other genital organs. From the anterior end of the prostate gland a duct passes obliquely just posterior to the pharynx, presumably to enter the genital atrium, but in this region details are obscured. The genital atrium opens to the exterior by a laterally placed genital pore. It was not possible to make out a penis such as is described by Folda (1928) for *M. marginata*. Just behind the testes lie the pair of oval bodies, organs of problematic nature, in which no cell boundaries are visible other than the investing membrane.

The pyriform receptaculum seminis is situated on the left of the vesicula seminalis (viewing the animal from the dorsal side), the anterior end passing forwards as the vagina to the vaginal pore on the lateral body margin, directly posterior to the genital pore. It was not possible to observe any communicating duct between the receptaculum seminis and the yolk reservoir.

The median and spherical ovary lies a short distance in front of the testes. The oviduct arising from the anterior border is directed anteriorly, and shortly after its inception receives a narrow duct from the large yolk reservoir on the left. It then passes to a more dilated portion which is possibly the same as the "pulsating organ" of Folda. Entering this structure are the ducts of scattered unicellular glands collectively forming the shell gland. From the distal end of the pulsating organ a duct leads out, passing at first anteriorly, and then, on reaching the level of the pars prostatica, runs obliquely across the animal and parallel with the male duct towards the genital atrium.

The vitellaria, consisting of very numerous small follicles, some irregular in shape, but chiefly oval, occupy all the space lateral to and behind the genital organs except for a small triangular section at the posterior extremity of the body. In addition the follicles extend equally on both sides of the body anteriorly to a short distance in front of the mouth opening. The vitelline

ducts have the usual disposition, the transverse duct from either side meeting in a very large yolk reservoir slightly displaced to the left.

Neither the excretory nor the nervous system were discernible in this specimen.

Discussion

The striking distinction between *Megalocotyle* and *Epibdella* as pointed out by Folda (1928), lies in the character of the posterior disc. This organ bears no septa in *Epibdella* and carries a simple marginal membrane, while in *Megalocotyle* it is traversed by a number of raised septa, and the membrane is thrown into wavy folds. On this feature Folda makes the genus *Megalocotyle*. The form here described agrees with *M. marginata* as regards the structure of the cotylophore, and most other characteristics. The difference, however, in the size and shape of the body, and the structure of the hooks on the posterior disc are considered sufficient justification for the erection of a new species.

Family Monocotylidae

Calicotyle inermis n.sp. (Pl. III, fig. 6)

Marine form, found in pouch-like dilatations of the oviducts of the female of *Pristiophorus cirratus* (Saw-shark).

Locality. Port Philip Bay, Victoria.

Incidence. About 75 per cent. of the female fishes examined were found to be harbouring two or three of the parasites.

Length of formalinised specimens, from the anterior end to the base of the cotylophore, varying from 8 to 11 mm. Maximum breadth towards the posterior end of the body 5·5–7 mm. Average diameter of cotylophore 3 mm. Ratio of total length of animal (*i.e.* including the cotylophore) to breadth, about 2 : 1. Cuticle unspined, but in some specimens the surface of the body appears to be covered with minute papillae measuring approximately 0·011 mm. in length. Since such papillae are not visible on all specimens it seems probable that they are retractile. Goto (1895), describing similar structures for *Tristomum sinuatum* and *T. ovale*, suggests they may be tactile in function.

When taken from the pouch the worms in life possess a beautiful pink to red coloration, and are characteristically curled, the anterior and posterior extremities being almost contiguous. The dorsal surface is convex, and the cotylophore, pressed against the concave ventral surface, is partly covered by the closely approximated wavy lateral margins. In the flattened specimens the cotylophore projects about one-half its diameter beyond the posterior body rim. Its eight loculi are typically arranged and more or less equal in size, thereby resembling *Calicotyle kroyeri* rather than *C. mitsukurii* or *C. australis*. In *C. inermis*, however, there are no hooks on the posterior disc; instead the surface is completely covered with minute papillae, more pointed

and longer (approximately 0·024 mm. in length) than those on the body surface. A further point of difference in this species is shown in the margin of the disc which is recurved over the concave ventral surface and broadly indented over each of the radial septa.

The mouth lies a short distance from the snout-like anterior extremity and is surrounded by the vestigial anterior sucker, in structure similar to that described by Wierzejski (1877) for *C. kroyeri*. The mouth likewise opens through a raised up swelling of the anterior wall of the sucker, while at the base of the large bowl-shaped muscular pharynx and across the short oesophagus and the bifurcation of the intestine lie the numerous so-called salivary glands typical of *Calicotyle* species. No cephalic glands, however, are discernible in the form here under discussion.

The intestinal limbs curve in characteristically towards the median line at the level of the ovary, usually making one or more similar undulations as they pass posteriorly, but such curves are by no means constant for the species.

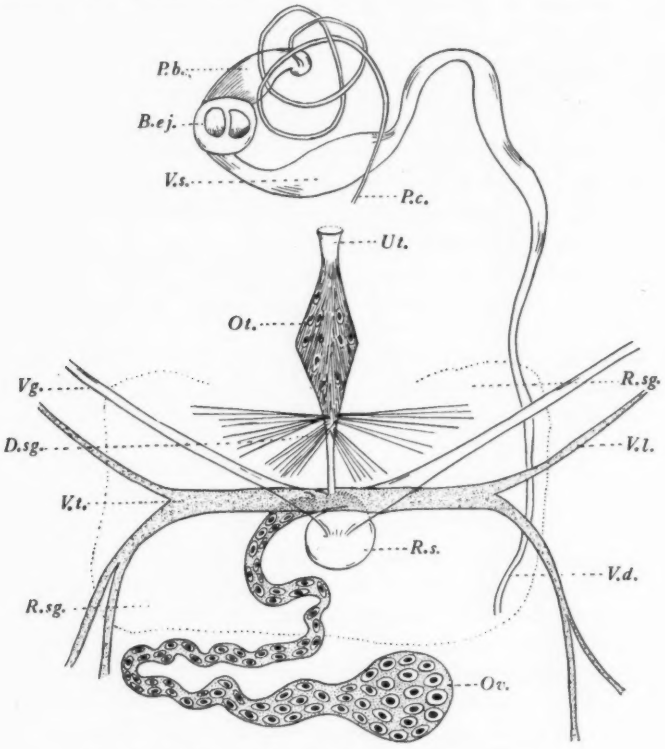
The main excretory canals lie just lateral to the intestinal limbs, while the excretory pores open to the exterior on the dorsal surface about the level of the bifurcation of the intestine. No pulsating bladder was observable even in living specimens.

The nervous system, in its dorsally situated brain and such of the main nerve trunks as are discernible, resembles completely the descriptions given for other species.

With the exception of the small, irregular-shaped and numerous testicles which lie scattered in the posterior half of the body between the intestinal limbs, the genital system is situated in the anterior one-third of the body. The vas deferens in this species, as in the type, passes anteriorly, running parallel with the right intestinal limb (viewed from the ventral surface), but slightly displaced towards the median line. At a short distance posterior to the bifurcation of the intestinal limbs the vas deferens curves inwards and dilates to form the vesicula seminalis (Text-fig. 1). The associated ejaculatory apparatus is similar in structure to that of *C. kroyeri*; the narrow chitinous penis, however, is considerably longer, measuring from 1–1·8 mm. in length.

The female system also offers only a few minor points of difference from that of other species. The long narrow ovary is typical in form and position, as are also the oviduct, receptaculum seminis, vitellarium, and the paired vaginae with their refractive membranous walls and the numerous unicellular vaginal glands. The long ducts of the unicellular glands, however, collectively constituting the shell gland, as will be seen from Text-fig. 1, enter the distal end of a short narrow tube and the proximal end of a thick walled spindle-shaped structure, in four or five groups. In *C. australis* (Johnston, 1934) the ducts on the other hand enter the proximal portion of the narrow tube. Consequently the expanded spindle-shaped structure into which the tube leads has been termed the uterus by Johnston. Here, however, although apparently an identical structure, it seems preferable to regard it as the ootype

as Goto has done for *C. mitsukurii*, and to regard as the uterus the narrow and thin-walled section into which the thick-walled section leads as the female tract passes to the median genital pore.



Text-fig. 1. *Calicotyle inermis*. Genital tracts, diagrammatic.

Discussion

Of the known species of *Calicotyle*, descriptions of which are available here, the above-described form differs chiefly in its larger size, in the absence of hooks on the cotylophore, in the absence of cephalic glands, and in the greater length of the chitinous penis.

Family Microcotylidae

Microcotyle sillaginae n.sp. (Pl. III, fig. 7)

Marine form, found on the gills of *Sillaginoides punctatus* (bay whiting).

Locality. Port Philip Bay, Victoria.

Incidence. Five specimens of the above species were found on the single fish examined.

Total length of formalinised specimens fluctuating about 4 mm.; breadth (max.) from 0·4 to 0·5 mm. Body slender, fusiform, symmetrical. Cotylophore distinctly demarcated from the body, encroaching on it, however, for at least one-half of its own length which is rather more than half that of the total length of the animal. Posterior suckers on the cotylophore typically consisting of small rectangular bags attached by the dorsal surface to its margin, and supported by the characteristic chitinous[1] skeleton of five pieces, an unpaired central and two pairs of lateral pieces (Text-fig. 2). The anterior arm of the U-shaped central piece is sharply bifurcated terminally, and longer than the posterior arm. The latter, however, is broader, and possesses a triradiate tip, the central ray of which is longer but less strongly chitinised than the laterals. The suckers vary little in size, the largest, 0·07 mm. in width, being found towards the middle of each row, the smallest, 0·05 mm., at the extreme posterior end. The number in this species is fairly constant, usually about thirty-two pairs.

Text-fig. 2. *a*, chitinous skeleton of posterior sucker of *Microcotyle sillaginae*; *b*, termination of posterior arm of central U-shaped piece of skeleton of posterior sucker of *M. victoriae*.

The anterior suckers are oval with comparatively thick walls and are situated typically one on each side of the buccal cavity into which they open on their inner sides. The major axis of each runs obliquely to the long axis of the worm. The average length of this axis is 0·08 mm., while that of the short axis is 0·04 mm. The membranous septum across the minor axis of the sucker, present in some species, is also to be observed here. In addition, from the base of the posterior side of the ellipse there is a further structureless membranous outgrowth, running parallel with that side and bordered with a single row of very fine small hooks.

At the anterior end of the animal no "sticky" glands, characteristic of most members of the genus, are here discernible; and there are no dorsal suckers.

The funnel-shaped mouth situated ventrally is subterminal, while the pharynx, small and spherical, leads into a comparatively short narrow tubular oesophagus.

It was not possible to follow the course of the limbs for more than a short distance, posterior to the bifurcation of the intestine in any of the specimens available.

[1] The term "chitinous" is used here simply in accordance with the usual custom adopted in this type of descriptive work.

The genital system typically occupies the central region of the animal. The eleven testicles, mainly paired, are separated from one another by a thin layer of connective tissue, and occupy a considerable part of the post-ovarian space (about one-third of the body length) towards the posterior end of the body. The vas deferens proceeds medially, dorsally to the uterus, as a tube with a structureless wall until it reaches within 0·4 mm. of the genital pore, when the wall becomes greatly thickened, muscular and slightly coiled. In one specimen the proximal end of this differentiated portion appears swollen and bulb-shaped. On reaching the small conical genital atrium the male duct passes through a papilla 0·027 mm. in height, after which, for the terminal portion of its length, approximately another 0·027 mm., the wall of the duct is chitinised to form a vestigial penis. The genital atrium is unarmed, ventrally situated, and its base is about 0·34 mm. from the anterior end of the animal. The common genital pore is not very distinct in any of the specimens to hand, but appears to be towards the apex of the atrium.

The ovary is a single, rather ill-defined but slightly coiled mass lying immediately anterior to the testes. A short oviduct passes posteriorly from the lower border of the right bend of the ovary, and receives first the genito-intestinal duct, and shortly afterwards the unpaired vitelline duct. In this species the ootype is a short narrow tube, and the shell gland, consisting of a number of large rounded to pyriform cells which stain darkly, is not bounded by any investing membrane. No receptaculum seminis is discernible.

The uterus, here a wide tube, proceeds medially to the female pore opening to the atrium a short distance posterior to the male opening.

The two lateral regions of the vitellarium in this species do not remain distinct throughout their length, but meet posteriorly behind the testicles, completely filling the extreme posterior region of the body proper. Anteriorly the irregular-shaped follicles extend to the level of the genital atrium.

Dispersed in groups among the follicles are numerous pigment granules similar to those recorded by other investigators. Some of the granules extend anteriorly as far as the pharynx, while posteriorly they are to be observed some distance along the length of the cotylophore.

The vaginae at their inception are paired, their unarmed dorsal openings being situated on either side about half-way between the lateral border and the median line, at about 0·4 mm. from the anterior extremity.

In some of the specimens the vaginae are considerably swollen with sperms and consequently conspicuous, but when empty they are narrow with apparently structureless walls, difficult to trace. The canals proceed obliquely and posteriorly, uniting in the median line to form a single tube of very variable length. This region of the body appears to be considerably influenced by the state of post-mortem contraction and consequently makes difficult the interpretation of the relation between vaginae and yolk ducts. In a specimen where conditions are most clearly shown, the proximal end of the canal appears to unite with the transverse vitelline ducts which have joined together, slightly

anterior to their origin. Posteriorly, the longitudinal vitelline ducts unite in the median line to form an unpaired duct which passes back to meet the oviduct.

Discussion

Microcotyle sillaginae in its general character shows considerable resemblance to *M. centrodonti* (Brown, 1929), but differs from that form in the smaller number of posterior suckers and testicles, in the absence of dorsal suckers and the paired vaginal openings. Brown questions the occurrence of the latter feature in *M. alcedis* and *M. canthari* (Parona and Perugia). Nevertheless, their presence in the form here under discussion is unmistakable; and further, *M. australis* (Murray, 1931) is also reported as having paired vaginal openings. Goto's definition of the genus must therefore necessarily be modified to accommodate such species as these, even should Parona and Perugia's interpretation of the openings in their species prove incorrect.

From the other known species *M. sillaginae* differs in one or more of the following features: absence of atrial spines, absence of dorsal suckers, character of cotylophore and number of posterior suckers in the paired vaginal openings, or finally in the number of testicles.

Microcotyle bassensis (Murray, 1931)

Total length of formalinised specimens, kindly lent by Miss Murray for re-examination, varying from 5·8 to 6·3 mm. Maximum breadth varying from 0·6 to 1 mm. according to the state of post-mortem contraction. The cotylophore, which has been described as constituting the posterior third of the animal, bears fifty to fifty-five pairs of small suckers, the chitinous framework of which consists of the five typical pieces. The largest of the suckers measure about 0·08 mm. and are found towards the centre of each row; the smallest measure 0·05 mm., and these are found generally towards the extreme posterior end, but some small ones are to be seen near the body. The oval buccal suckers measure from 0·08 to 0·1 mm. along the major axis, while 0·04–0·08 mm. is the variable length of the minor axis.

The testicles, which are characteristically post-ovarian, occupy entirely the most posterior portion of the central body space (approx. 0·9 mm. in length), and vary in number from thirty to thirty-six. The median vas deferens opens to the genital atrium through a small rounded papilla, the pore lying just posterior to the more ventrally situated uterine pore. The genital atrium is divided into two parts. The lower, receiving the openings of the genital tracts, has a few laterally placed spines; the upper occupied solely by numerous atrial spines continuous with the lower ones. These spines are small, conical, slightly curved structures, the largest measuring approximately 9μ. The ventral surface above the genital atrium appears as a rounded swelling in the centre of which lies the common genital pore.

The vitellaria of either side do not unite at the posterior end of the body but characteristically terminate lateral to the most posterior testicles.

As in *M. sillaginae*, some pigment granules appear along the median line of the cotylophore.

Microcotyle victoriae n.sp.

Marine form, found on the gills of *Helicolenus percoides* (red gurnet).

Locality. Port Philip Bay, Victoria.

Incidence. These parasites are by no means of frequent occurrence. Generally present in twos or threes, out of a large number of fish examined only about one-third harboured any parasites.

Total length of formalinised specimens varying from 3·80 to 5·40 mm., with an average length of 4·82 mm. Maximum breadth from 0·70 to 1·10 mm., with an average of 0·87 mm. Body slender, symmetrical. Cotylophore about one-quarter the length of the whole body, and distinctly separated from it. Posterior suckers, from seventeen to twenty-five pairs, most specimens however having about twenty-one pairs. Size as in *Microcotyle bassensis*. The associated chitinous framework varies slightly from that of the other species, the termination of the shorter posterior arm being broadly spatulate (Text-fig. 2 *b*). Dimly discernible there is a triradiate tip as in *M. sillaginae*, but it is apparently not similarly chitinised.

The anterior suckers in this species are almost circular, and not so obliquely placed as in *M. sillaginae*. The axes in the smaller specimens measure about 0·08–0·06 mm., but the average measurements are 0·1 mm. for the major axis and 0·08 mm. for the minor. A membranous septum is present across the minor axis, but there are no rows of small hooks at the base of the suckers.

At the extreme anterior end of the animal are the three characteristic groups of "sticky" glands, here comparatively large and typically situated, the most anterior group being median, the other two lateral. There are no dorsal suckers.

The excretory pores lie on the dorsal surface near the lateral body margin of either side, about 0·44 mm. from the anterior extremity.

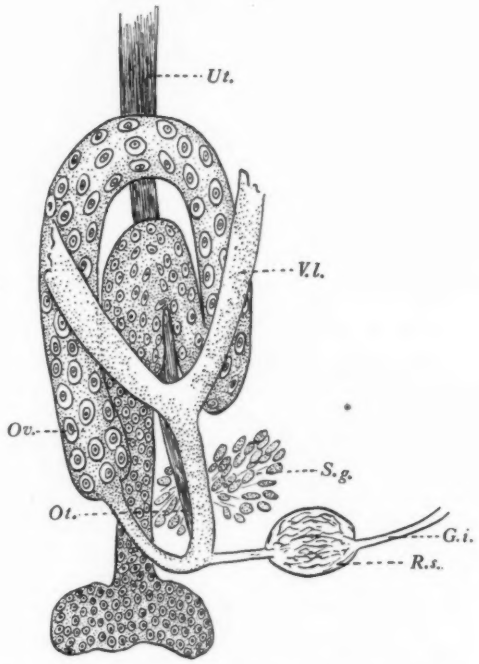
The subterminal ventral mouth is rather slit-like with a folded ventral lip. The pharynx is small and spherical, while the oesophagus is comparatively narrow and tubular. The intestinal bifurcation occurs just dorsal to the lower half of the genital atrium. The limbs are of unequal length, the longer passing down into the cotylophore.

The testicles in this species are small and rounded, numbering from eighteen to twenty-two, and filling almost completely the extreme 0·8 mm. of the posterior region of the body.

The ovary (Text-fig. 3) is characteristically coiled, and the associated ducts and their connections are typical. There is a small spherical receptaculum seminis from which leads out a short genito-intestinal canal, and the shell

gland surrounding a narrow tubular ootype consists of numerous small unicellular glands not surrounded by an investing membrane.

The vagina is a single median canal opening to the exterior on the dorsal surface about 0·9 mm. from the anterior end of the animal. It passes posteriorly a variable distance, depending on the state of contraction of the individual specimen, but averaging about 0·7 mm. from the pore, before dividing into two to meet the paired yolk ducts.

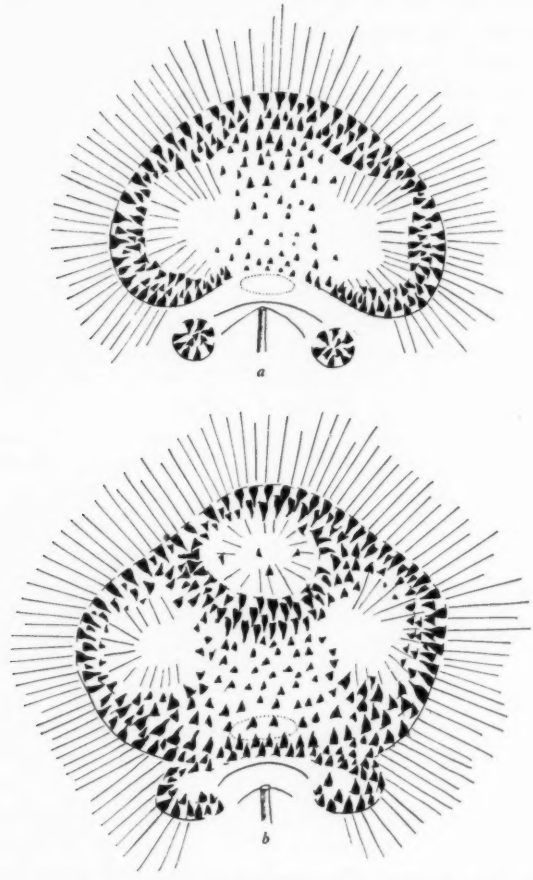


Text-fig. 3. *Microcotyle victoriae.* Female genital organs, diagrammatic.

The two lateral regions of the vitellarium in some specimens remain distinctly separated at the posterior end, but in others a few follicles lying posterior to the testicles connect up the two sides.

The genital atrium in this species resembles that of *M. bassensis* although slightly smaller, and the pores are similarly situated, but the arrangement of the atrial spines is rather different (Text-fig. 4). The spines, which measure from 5 to 9 μ, are definitely divided into two sets, corresponding to the divisions of the atrium, those lying lateral to the male and female openings in the posterior portion being quite distinctly separated from those in the more anterior part of the atrium, and consisting of a small rosette of not more than twelve spines on either side. The upper group of spines lie chiefly on the

lateral and ventral walls of the atrium, the whole presenting a reniform shape, in the hilus of which the common genital pore opens to the exterior on the ventral surface.



Text-fig. 4. Arrangement of atrial spines: *a*, in *Microcotyle victoriae*; *b*, in *M. bassensis*. In both cases the male opening is the proximal, the uterine opening is shown just anterior to it, and the common genital opening by the dotted ring.

Discussion

The new species *M. victoriae* is closely related to at least five species of the genus, namely *M. australiensis* (MacCallum, 1921), *M. sebastis* and *M. elegans* (Goto, 1895), *M. bassensis* (Murray, 1931), and *M. hiatulae* (Goto, 1899).

Concerning *M. australiensis*, according to the data given, that form is a

A.s.
P.c.
Mth.
Sal.
Ut.
Ov.
Tt.
S.g.
Ov.
Ut.op.
Vo.
Vg.
G.a.
Vd.
Ut.
V.m.
Ot.
Vd.
Tt.
Pr.O.
Ph.
Pg.
Ut.
Po.
Ov.
G.p.
Vo.
V.s.
R.s.
V.r.
Vd.
T.
1
2
3
4
5
6
7

smaller parasite with a slightly larger number of testicles and a comparatively larger cotylophore. Owing, however, to lack of other anatomical details, no further comparison can be made.

M. victoriae differs from *M. sebastis* and *M. elegans* in its comparatively smaller cotylophore and in the possession of fewer posterior suckers and fewer testicles. In addition, it differs from *M. sebastis* in the size of the atrial spines, while from *M. bassensis* it differs in the arrangement of these organelles. *M. victoriae* most closely approaches *M. hiatulae*, but there is a considerable difference in the arrangement and length of the atrial spines.

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EXPLANATION OF PLATE III

Fig. 1. *Megalocotyle helicoleni* n.sp. Whole specimen, dorsal view.
Fig. 2. *Megalocotyle helicoleni*. Single hook of most anterior pair on cotylophore.
Fig. 3. *Megalocotyle helicoleni*. Different views of middle pair.
Fig. 4. *Megalocotyle helicoleni*. Single hook of posterior pair.
Fig. 5. *Megalocotyle helicoleni*. Different views of marginal rods.
Fig. 6. *Calicotyle inermis* n.sp. Whole specimen, ventral view.
Fig. 7. *Microcotyle sillaginae* n.sp. Whole specimen, dorsal view.

Key to Lettering

A.s. anterior sucker; *B.ej.* bulbus ejaculatorius; *D.sg.* ducts of unicellular glands constituting shell gland; *G.a.* genital atrium; *G.i.* genito-intestinal canal; *G.p.* common genital pore; *Mth.* mouth; *Ot.* ootype; *Ov.* ovary; *P.b.* bulbus penis (bulbus copulatorius); *P.c.* chitinous penis; *P.g.* prostatic gland; *Ph.* pharynx; *P.o.* pulsating organ; *Pr.O.* problematic organ; *R.s.* receptaculum seminis; *R.sg.* region of shell gland; *Sal.* salivary glands; *S.g.* shell gland; *T. testis*; *Tt.* testicles; *Ut.* uterus; *Ut.op.* uterine opening; *V.d.* vas deferens; *Vg.* vagina; *V.l.* longitudinal vitelline duct; *V.m.* median vitelline duct; *V.o.* vaginal opening; *V.s.* vesicula seminalis *V.t.* transverse vitelline duct; *Y.r.* yolk reservoir.

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STUDIES ON POPULATIONS OF HEADLICE (*PEDICULUS HUMANUS CAPITIS: ANOPLURA*). I

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Many biologists are at present interested in populations of animals, which are studied experimentally and also by more abstract mathematical methods, but it is frequently difficult to obtain facts about actual populations living under natural conditions unless they are studied by sampling, the accuracy of which cannot always be precisely defined. The method described in this paper tells us of the actual numbers of headlice in crops of hair on individual people. From it we may learn the proportion of people who are infested at different times or places, and the number of lice on different people: also the proportion of males, females and young in the louse population. The results with material from Woolwich and from Lagos are set out in this paper. Further work is now in progress on material received from several parts of the world: this material will be studied and the results published at a later date.

Available information on the prevalence of lice in human populations was summarised by Nuttall (1917 *b*). Most of it relates to body lice (*Pediculus humanus corporis*): with regard to this subspecies Nuttall gathered together a number of facts about the proportion of human beings who are infested in different parts of the world and in different social circumstances. He could find no actual enumerations of headlice, but published a single count made by himself.

I. Methods

The general method is to obtain the crop of hair of a large number of individuals, to dissolve each sample separately in alkaline sodium sulphide, remove the lice and count them.

The method of collecting depends on local circumstances, but the purpose is to obtain the whole crop of hair of a number of individuals separately, removing it from the head with a razor if possible. In some places one can obtain material from the subjects of post-mortems, in others from prisoners on admission to jail or from the poorer members of the community who are willing to be completely shaved for a small sum. As the methods of collection are different, the figures from different countries are not strictly comparable,

but care has been taken to see that no selection is made of the infested or clean, and that as far as possible the details of collecting are standardised and adhered to in each country. In this matter much depends upon the person who is good enough to supervise the collecting. The hair from each individual, as soon as it has been taken off, is put into an envelope; on the outside of the envelope the necessary facts are written. They vary slightly with local conditions, but one should at least have name, sex, approximate age, race and date. It is essential that the work should be carried out throughout the year, and at present we are inclined to think that about thirty specimens per month are sufficient.

Batches of envelopes are posted to London, where the specimens of hair are weighed and transferred to beakers containing the sulphide solution, which are set in a bath of boiling water. We find that the following solution gives satisfactory results:

50 g. potassium hydroxide.
100 g. sodium sulphide.
1 litre tap water.

Half a litre of this solution is sufficient to dissolve 30 g. of hair, which is roughly the crop of a European man. The hair dissolves completely in two to three hours, forming a black solution in which the lice cannot be seen. But they can be easily removed by passing the solution through a funnel of stainless steel gauze, sixty meshes to the linear inch: we find that this mesh takes out even the newly hatched lice. Any material which has not dissolved in the solution and which is retained in the gauze funnel is washed back into a dish and the lice are searched for and counted. With a mechanical counter in the left hand and a pipette in the right, it is easy to count the males, females and young separately. The males are easily picked out by the aedeagus, and the females by the posterior lobes and the gonopods (Nuttall, 1917 *a*; Keilin and Nuttall, 1919). No attempt has been made to keep a separate record of the numbers of the three nymphal instars. All the data are recorded on cards, one for each man.

While this method was being developed and standardised, several others were tried. Tests were carried out with different concentrations of alkalis and sulphide, and with different volumes of solution. I understand that it is necessary to keep the *p*H at 11·0 or higher, but the empirical method described above appears to give good uniform results. Attempts were made to filter the solution, which appears to be colloidal, through very coarse filter paper and were abandoned when it was found that the wire gauze gives very clean, quick results. It was also discovered that the lice could be removed from the sulphide solution by differential flotation. If the solution of hair is shaken up with a considerable volume of liquid paraffin, some of the lice are wetted all over with the paraffin and float up into it when the vessel is allowed to stand. Others are partly wetted with the paraffin and accumulate in the layer separating the two liquids. The aqueous solution can be run off from below

and the lice counted, but the method is dirty and tedious and not to be compared in efficiency with the one that has been described.

Several general objections should be considered. It is clear that the results depend greatly on the person who supervises the collection of the material, and it is not easy for him to ensure that the barber and the clerk continue to carry out their work regularly and carefully. It is possible also that after the material has been collected some lice migrate away from the hair, but it seems that they nearly always continue to clasp a hair until death for we have never found them dead on the inside of the envelope or loose in it. There is also the possibility that some of the lice are carried off by ants in certain tropical countries; against this we cannot guard. Early in the investigation we feared that cast skins might be numerous in the hair and might readily be mistaken for lice, but they are easy to distinguish. For a short period all the cast skins, even fragments, were recorded; the proportion of them to lice varied greatly on individual heads, but on an average we found one cast skin for five lice.

It seems clear that the method here described could be used for many other types of experimental work. The solution dissolves not only hair and feathers but also skin: if the skin is removed from a mouse or canary, the lice on the animal can easily be counted. One might therefore investigate the spread of lice through a community of animals most of which had originally been clean, or the effect of nutrition upon infestation.

It is a pleasure to acknowledge the careful work of my assistant, Mr S. Smith, who has been responsible for the large amount of the routine work and counting, which is the basis of this paper.

II. Data from Woolwich, England

I have found great difficulty in obtaining suitable material from Great Britain, but owing to the kindness of the Director-General of Medical Services at the War Office it was arranged that Major F. C. Tibbs, R.A.M.C., should send me a regular supply of clippings from recruits admitted into the Woolwich Depôt. The hair of these men is removed with scissors to a standard degree of shortness, but is not cut very close to the scalp; as it is known that lice when they are present are generally found close to the skin, the figures are not comparable with those which would be obtained by shaving the whole scalp. Altogether 235 specimens have been received during all the months of the year ending April 1935. No lice were found in any of the specimens. For the reason just given, we cannot feel certain that the insects are entirely absent from all these individuals, but it seems clearly indicated that if any infestations occur in young men of this class they are rare and light.

III. DATA FROM LAGOS, WEST AFRICA

Material. The material was collected from dead bodies, subjects of post-mortem examinations in Lagos, from February 1934 to February 1935. The hair of the whole scalp was removed with a razor; no selection of clean or lousy people was made, though I believe that the hair was not removed from everybody which came up for examination. All the people whose hair was examined were African by race. I am very much obliged to Dr J. Cauchi, who obtained and forwarded the specimens.

Crude figures. The crude figures are shown in Table I. It will be observed that of the heads examined, almost exactly 20 per cent. harboured lice. Moreover, the frequency distribution among those found infested is very peculiar, the number of lice ranging from one to 1286.

Table I. *Showing the prevalence of lice in Lagos, in the heads of people of many African races, and both sexes*

Month	Total heads	No. lice per head			
		Nil	1–10	11–100	101 and over
1934 Feb.	3	2	—	1	—
Mar.	16	12	1	2	1 (263)
Apr.	12	10	1	1	—
May	14	10	1	3	—
June	9	7	1	1	—
July	10	9	—	—	1 (131)
Aug.	10	8	—	—	2 (368, 130)
Sept.	8	5	2	1	—
Oct.	5	4	—	—	1 (1286)
Nov.	6	6	—	—	—
Dec.	3	3	—	—	—
1935 Jan.	5	4	1	—	—
Feb.	1	1	—	—	—
Total	102	81	7	9	5

Sex of human being. In tabulating the material it was observed that women were more frequently and more heavily infested than men. This is shown very clearly in Table II: "all races" include people from many parts of Nigeria (several Hausas), also Liberians and men from Togoland, Sierra Leone, etc. "South Nigerians" are Yoruba, Ijaw, Sobo, etc., also people from Warri, Benin, Calabar.

Table II. *Showing the prevalence of infestation in the heads of men and women separately*

Race	Sex	Total heads	No. lice per head		
			Nil	1–100	101 and over
All races	♂	77	67	7	3
„	♀	25	14	9	2
South Nigerians	♂	46	40	5	1
„	♀	22	12	9	1

The difference between men and women is considerable, and the appropriate statistical test (χ^2) shows that it is significant; for the differences recorded between males and females (both in all races and in South Nigerians) would occur less than one time in a hundred if they were due to chance. It seems probable that the greater lousiness of the women is due to their elaborate and rather permanent head-dressing. But it is possible that the women are more infested than the men because their hair is longer: the difference is considerable, for the average weight of a man's hair is 6–7 g., of a woman's 24 g. But in spite of a considerable amount of work, I have not established any statistical relation between the weight of a crop of hair and its infestation.

Race. It is generally held in Nigeria that Hausas and men from the north are more heavily infested than men from the south (at least with body lice). But so far as Hausas resident in Lagos are concerned, I have the following figures:

Race	Sex	Total heads	No. lice per head		
			Nil	1–100	101 and over
South Nigerian	♂	46	40	5	1
Hausa	♂	18	14	2	2

Inspection seems to show that there is no great difference in the rate of infestation in the two samples. If the difference is tested statistically (by χ^2), it can be shown that it is not significant, *i.e.* the above difference might frequently be obtained if there was no difference between the races and if one examined a number of samples of that size. It will, of course, be realised that these Hausas had perhaps lived long in Lagos: they give no indication of what might be found on Hausas in their own country.

Season. The climate of Lagos is equable and of the equatorial type. The seasonal differences in temperature are very slight indeed. The means (half the sum of the maximum and minimum) of the hottest and the coldest months in 1934 were 83·1 and 78·9° F. (28·4 and 26·1° C.) respectively: it is therefore unlikely that there is any effective change in temperature on the surface of the human head since the atmospheric temperature is so equable. The humidity also is high and constant: the monthly means for 1934 (obtained by averaging the readings at 9 and 15 hours) run from 73 to 85 per cent. But the rainfall shows much more definite seasonal changes, the winter months being nearly dry and the summer months very wet. In the thirteen months under review, from February 1934 to February 1935, the monthly totals exceeded 5 in. in the five months from April to August and were below 5 in. in the other months except that 13·45 in. was registered in October. In the crude figures given in Table I it appears that lice are rarer in the dry season than in the summer, but differences due to sex are so considerable that the crude figures must be disregarded. I have therefore brought together the figures for all males, which are as follows:

Period	Total heads	No. lice per head		
		Nil	1–100	101 and over
Apr.–Aug. inclusive	41	35	5	1
Other months	36	32	2	2

One may conclude from this that there is no significant difference between the two seasons. I have also considered the figures for South Nigeria, males only, a smaller but more homogeneous group of people. Here again no significant difference is discoverable. We may conclude that there is no evidence in our figures of any seasonal distribution in infestation at Lagos. This is not unexpected, because the climatic conditions are so equable.

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MORPHOLOGICAL AND TAXONOMIC STUDIES ON MAMMALIAN TRYPANOSOMES

I. THE METHOD OF REPRODUCTION IN ITS BEARING UPON CLASSIFICATION, WITH SPECIAL REFERENCE TO THE *LEWISI* GROUP

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(With 1 Figure in the Text)

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Introduction

There has always been a considerable amount of controversy regarding the classification of trypanosomes, due mainly to lack of agreement in the choice of criteria for the identification and differentiation of the vast number of these parasites known at present, distributed among a multiplicity of hosts and varying considerably in their effect upon the latter, though differing only slightly in morphology.

This problem would present less difficulty if the classification of trypanosomes were dealt with purely from the zoological point of view. However, the medical and veterinary importance of these parasites, though responsible for most of our knowledge concerning them, has at the same time encouraged their systematic treatment by workers very often unfamiliar with or neglectful of the accepted rules and traditions of taxonomy.

As the result of such treatment the classification of trypanosomes is in many respects artificial and does not always reflect their true affinities. Many forms indistinguishable morphologically have been given independent specific status merely on the grounds of physiological differences between them, and though the number of such species has been somewhat reduced in recent years, even in its more generally accepted form the classification of trypanosomes represents a compromise between the strict requirements of zoological taxonomy and the considerations of practical convenience.

Practically all the known trypanosomes are referred to the genus *Trypanosoma* Gruby, 1843, with the exception of one constituting the genus *Endotrypanum* Mesnil and Brimont, 1908, and one or two others placed in the genus *Schizotrypanum* Chagas, 1909.

Nothing can be said at present regarding the position of *Endotrypanum* which is a *genus inquirendum*, its sole representative, *E. schaudinni*, standing in need of further investigation.

As regards *Schizotrypanum*, some authors recognise its independent status, while others prefer to retain the type species, *S. cruzi*, in the genus *Trypanosoma*. There are other authors again who use the two generic names indiscriminately when speaking about this trypanosome.

The most complete presentation of the case in favour of the recognition of *Schizotrypanum* was recently made by Dias (1934*a*). My own views have been defined in several recent publications (Hoare and Coutelen, 1933; Hoare, 1934, 1934*a*, 1935) in which the retention of *S. cruzi* in the genus *Trypanosoma* is advocated. In discussing the opinions of the supporters of the latter point of view, Dias (1934*a*, p. 223) makes the following comment: "Un des auteurs qui c'est avec le plus d'assurance prononcé sur la question, c'est....Hoare [1934], qui, toutefois, n'a pas tenté de recourir à des arguments bien fondés.... En posant catégoriquement en fait la validité exclusive de ce genre [*Trypanosoma*], l'auteur ne tient nul compte de la diversité des opinions et des raisons qui viennent à l'appui de celles qui lui sont contraires."

As a matter of fact, in adopting this attitude I merely ranged myself on the side of those authors who take the conservative point of view, not deeming it necessary to enter into a lengthy discussion of the subject. It must be admitted, however, that the case for *Trypanosoma* was never argued *in extenso*, its adherents accepting the *status quo ante* 1911, when Chagas himself reverted from the emended *Schizotrypanum cruzi* (Chagas, 1909) to the original name *Trypanosoma cruzi* Chagas, 1909 (cf. Hoare, 1934*a*).

Dias' paper has brought the matter to a point when a revision of this question has become necessary, and since the arguments advanced by him have not modified my own views on the subject, and as, moreover, he has to a certain extent placed upon me the *onus probandi*, I propose to set forth in this paper the reasons which lead me to regard the parasite causing Chagas' disease as a member of the genus *Trypanosoma*.

The views of previous authors on this question have already been adequately reviewed by Dias (1934*a*). Since they have not only been fully assimilated but considerably amplified by this author, they need not be considered separately here.

DEFINITION OF *SCHIZOTRYPANUM*

According to Dias (1934*a*, p. 218): "L'absence de multiplication dans le sang périphérique à la manière des autres trypanosomes, et l'exsitence d'une phase intra-cellulaire d'évolution et de multiplication, constituent la carac-

téristique principale, et par là, la principale raison de reconnaître les parasites qui possèdent cette caractéristique, comme formant un groupe ou genre à part [=*Schizotrypanum*] distinct de celui des véritables trypanosomes." Moreover, it is pointed out that Chagas' trypanosome differs from the other trypanosomes in that its reproduction takes place in the leishmanial stage and not in the trypanosome stage. The bat trypanosome, *S. pipistrelli*, "qui ne se divise pas non plus dans le sang", is associated with *S. cruzi* in the same genus.

On the grounds that *S. cruzi*, in the course of its development in the vertebrate, "assume alternativement les caractéristiques morphologiques des genres *Leishmania* et *Trypanosoma*", Dias (1934*a*, p. 224 *et seq.*) assigns to *Schizotrypanum* an intermediate position between the two last-named genera. In accordance with these views he proposes the following classification for the Trypanosomidae of vertebrates:

(*a*) Flagellates which multiply *in the tissues* of the vertebrate in the *leishmanial stage*:

(1) Flagellates with leptomonad stage in the insect-vector and leishmanial in the vertebrate: Genus *Leishmania*.

(2) Flagellates attaining trypanosome stage in both hosts: Genus *Schizotrypanum*.

(*b*) Flagellates without intracellular development in the vertebrate, multiplying *in the blood* in the *trypanosome stage*; attaining trypanosome stage in intermediate host: Genus *Trypanosoma*.

It will be seen that the new classification introduces radical changes in our conceptions of the Trypanosomidae as a whole, for it not only recognises the independent status of *Schizotrypanum*, but widens the gap between this genus and *Trypanosoma* still further by uniting it with *Leishmania* into a group placed in opposition to *Trypanosoma*.

The full significance and implications of this classification can only be properly appreciated if the whole group of trypanosomes is re-examined from the point of view of comparative morphology. A critical analysis of this kind will enable us to consider them in true perspective and to arrive at a correct interpretation of their affinities and systematic position.

Critical analysis of the systematic characters of *Schizotrypanum*

It is well known that there are no standard criteria for the definition of species and genera and that these categories are not equivalent throughout the various subdivisions of the animal kingdom. However, within the limits of a given group of organisms these systematic units have the same value, *i.e.* the degree of distinction between units of the same rank (genus or species) is of the same order.

In order to justify the separation of *Schizotrypanum* from *Trypanosoma* it is necessary to show that the characters which differentiate its repre-

sentatives from those of the latter genus are peculiar to it, and do not occur in other trypanosomes, and, moreover, that the gap separating it from them is considerably greater than those which separate the species of *Trypanosoma* from each other.

The evidence on which the differentiation of the genus *Schizotrypanum* is based is twofold: (1) *biological*, or rather *ecological*, which is concerned with the site in which the parasites develop, and (2) *morphological*, relating to the stage in which they reproduce.

As was shown above, the fact that the type species, *S. cruzi*, does not multiply in the blood of the vertebrate host, but in various other tissues, has been advanced as the main argument for the separation of *Schizotrypanum*. This argument is, however, inadmissible for several reasons. In the first place, it is a generally recognised principle, both in zoology and in botany, that the essential criterion in the constitution of a systematic unit is the morphology of the organisms, the classification of which is based on the difference of structural characters between them. The biological properties of the organisms are, from the taxonomic point of view, only incidental attributes of no systematic value, though they serve to precise the ecological or physiological peculiarities of the organisms and may provide supplementary characters of intraspecific value (*e.g.* in the case of "physiological races"). From this point of view there can be no justification for the separation of morphologically similar organisms on the grounds of difference in habitat.

The argument that *Schizotrypanum* differs from *Trypanosoma* in that the former reproduces in the "tissues" and the latter in the blood is, moreover, fallacious, amounting to the differentiation of these flagellates according to the type of tissue in which they develop, since histologically blood represents merely a variety of connective tissue in which the intercellular substance is liquid, while its constituent elements are genetically closely related to those of the reticulo-endothelial system, for which *Schizotrypanum cruzi* shows a particular predilection[1].

Furthermore, the histotropism of *S. cruzi* is by no means exceptional, since other trypanosomes also occur regularly and are capable of multiplication in the "fixed" tissues. Thus Peruzzi (1928) described large accumulations of leishmanial forms of *Trypanosoma brucei*, *T. rhodesiense* and *T. gambiense*, situated in the heart muscle of infected monkeys. Cellular invasion was likewise observed both in the cardiac muscle and in other histological elements. The leishmanial forms manifested definite signs of division, and it was concluded that "These enormous quantities of parasites are certainly the product of multiplication *in situ*", while their similarity to the tissue stages of *Schizotrypanum cruzi* is also noted. These findings and conclusions were later fully confirmed by Hoeppli and Regendanz (1930). Similar observations were recorded by Schwetz (1928) and Schwetz and Fornara (1928, 1929) who found

[1] Tissues of this type (blood, lymph) can be referred to as "fluid", while the other tissues may conveniently be called "fixed".

masses of leishmanial forms, some of them dividing, in the heart muscle of cattle and pigs infected with *Trypanosoma vivax* and *T. congolense*. Schwetz and Fornara (1929) noted that "Les amas de nos corps leishmanioïdes ressemblent beaucoup à ce qu'on trouve dans les organes des individus atteints de *Schizotrypanum cruzi*". It should also be noted that, in addition to the leishmanial stage, the polymorphic trypanosomes invade various organs of their host in the flagellate stage, and can therefore be regarded as true "tissue" parasites.

Dias (1934*a*) regards the facts described by Peruzzi as insufficient to invalidate the position of *S. cruzi*, on the grounds that they are not of general occurrence and have a different significance. Nevertheless, the observations quoted above are sufficient to indicate that invasion of the "fixed" tissues by trypanosomes and their multiplication in the leishmanial stage are found quite commonly in trypanosomes, and are not limited to *S. cruzi*. The difference in this respect between the latter species and others, being only one of degree, merely emphasises their close relationship. Whereas in *S. cruzi* multiplication within the "fixed" tissues in the leishmanial stage is a necessary phase of its development, in the pathogenic trypanosomes referred to above this method of reproduction does not appear to form an essential part of the life cycle, their multiplication proceeding chiefly in the blood, in the trypanosome stage.

On the other hand, binary fission of the trypanosome forms of *S. cruzi* in the blood—the absence of which in this trypanosome was advanced as a differential diagnostic character—occurs more commonly than has been previously assumed (Dias, 1934, 1934*a*). Thus Mazza and Romaña (1934) describe a case of Chagas' disease in which numerous dividing trypanosome forms were seen in the blood. These were depicted (cf. Fig. D, 3*a*), thus leaving no doubt as to the correctness of the interpretation.

The morphological distinction between the genera *Schizotrypanum* and *Trypanosoma*, according to Dias (1934*a*) and others, amounts to *reproduction in the leishmanial stage* in the former genus. However, the only other recognised species of this genus, *Schizotrypanum pipistrelli*, which multiplies in the inner organs of the bat, lacks this essential character, for *it reproduces not in the leishmanial stage*, but in the form of a crithidia, as demonstrated by Chatton and Courrier (1921). In view of this discrepancy it is necessary either to extend the definition of the genus *Schizotrypanum* by admitting into it trypanosomes which multiply within the "fixed" tissues in the *crithidial* stage, or to remove *S. pipistrelli* from this genus. However, as will be shown below, this flagellate does not differ in any essential way from the typical members of the genus *Trypanosoma*.

The assertion made by Dias (1934*a*) that flagellates of the genus *Trypanosoma* differ from those of *Schizotrypanum* in that the former reproduce in the vertebrate by binary fission in the trypanosome stage is also entirely misleading, for it is true only for trypanosomes belonging to the *evansi*, *vivax*, *congolense* and *brucei* groups (=section B in the classification of Hoare and

Coutelen (1933) adopted here). As regards the large and important *lewisi* group (=section A), in all the species in which the complete life cycle is known division in the trypanosome stage is just as exceptional as in *S. cruzi*.

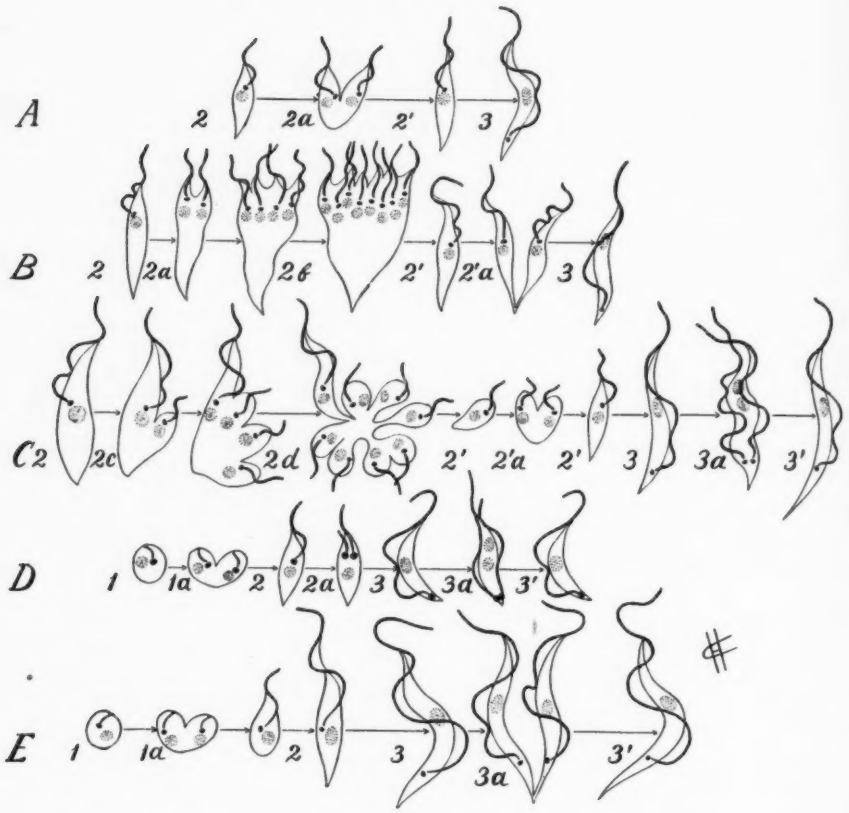
STAGES OF DEVELOPMENT OF THE TRYPANOSOMES

The stages of development of these trypanosomes in the vertebrate host have never been the subject of a comparative study, although they provide important morphological criteria for the differentiation of species, especially of those which are indistinguishable in the trypanosome stage (*e.g.* the rodent parasites).

With the object of throwing more light on the mutual affinities of these trypanosomes, I propose to give a brief account of their development. The stages of development of the trypanosomes of the *lewisi* group, including those referred to *Schizotrypanum*, are represented semi-diagramatically in the accompanying figure.

In the hamster trypanosome, *Trypanosoma rabinowitschi* (=*T. criceti*), the development of which has been worked out recently by Regendanz (1929) and by Nattan-Larrier and Noyer (1933, 1933*a*, 1933*b*), the multiplication takes place in the blood and lasts not more than 24 hours. Before proceeding to divide the trypanosome assumes the crithidial structure (Fig. B, 2) and remains in this stage throughout the entire period of multiplication. Reproduction is by a type of *equal multiple fission* (Fig. B, 2*a*, 2*b*) in the course of which multinucleate multiflagellate individuals are produced. The undetached daughter elements are similar in appearance and size, and the whole dividing form is bilaterally symmetrical, being campanulate or fan-shaped. On the completion of division the detached daughter individuals remain in the crithidial stage (Fig. B, 2′) and undergo equal binary fission (Fig. B, 2′*a*), giving rise to the typical trypanosomes (Fig. B, 3), which represent the established phase of the infection. As far as is known, the trypanosomes do not multiply any further.

Like the preceding species, the rat trypanosome, *T. lewisi*, develops in the blood of its host. Its period of multiplication is likewise restricted in time, but lasts several days, and in the course of reproduction the flagellate first assumes the crithidial form (Fig. C, 2). It also undergoes multiple fission, but, in distinction from the hamster trypanosome, the division in the rat trypanosome is *unequal*, viz. the parent form retains its individuality throughout the entire process, and can be distinguished from the daughter elements by its larger size and long flagellum (Fig. C, 2*c*, 2*d*), the dividing form being consequently asymmetrical in appearance. The daughter individuals represent leishmanial or short crithidial forms (Fig. C, 2′) which may divide further by equal binary fission (Fig. C, 2′*a*) and give rise to the trypanosome forms (Fig. C, 3) constituting the established blood phase. The trypanosome forms are capable of dividing by equal binary fission (Fig. C, 3*a*, 3′), but these stages are rarely encountered.



The life-cycle of trypanosomes of the *lewisi* group in the mammalian host.

A. *Trypanosoma pipistrelli* (= *T. vespertilionis*?). [Adapted from Chatton and Courrier (1921).]

B. *T. rabinowitschi* (= *T. criceti*). [Adapted from Regendanz (1929) and Nattan-Larrier and Noyer (1933).]

C. *T. lewisi*. [Original, and adapted from Minchin (1912) and Wenyon (1926).]

D. *T. cruzi*. [Adapted from Wenyon (1926), Dias (1934), and Mazza and Romaña (1934).]

E. *T. theileri*. [Original, and adapted from Carpano (1932).]

In each row are shown the successive stages of development of the flagellate in the vertebrate host. The forms on the extreme left represent the initial stage preceding the reproductive phase, those on the extreme right are trypanosomes representing the end stage of reproduction, or the established blood-forms. Stages of the same morphological type bear the same numbers:

1, leishmanial stage; 1*a*, binary fission of leishmania; 2, crithidia; 2*a*, equal binary fission of crithidia; 2*b*, equal multiple fission of crithidia; 2*c*, unequal binary fission of crithidia; 2*d*, unequal multiple fission of crithidia; 2′, crithidia resulting from fission; 2′*a*, equal binary fission of crithidia (2′); 3, 3′, trypanosomes: established blood forms; 3*a*, equal binary fission of trypanosomes.

As in the preceding two trypanosomes, the reproduction of *Schizotrypanum cruzi* in the vertebrate host is limited to a special period of multiplication followed by the established phase. It proceeds within the "fixed" tissues in the leishmanial stage, by *binary fission* (Fig. D, 1, 1*a*), the products of which later assume the crithidial form (Fig. D, 2). The crithidia also divide by binary fission (Fig. D, 2*a*), and from them arise the typical trypanosomes (Fig. D, 3) of the established blood phase. As has been mentioned already, these trypanosomes are capable of dividing further by binary fission (Fig. D, 3*a*), though, as in the case of *Trypanosoma lewisi*, this appears to be a rare phenomenon.

Our knowledge about the multiplication of *T. theileri* is incomplete, but from the observations of Carpano (1932) it would appear that the reproduction of the cattle trypanosome is similar to that of *Schizotrypanum cruzi*, namely it multiplies in the "fixed" tissues (brain and lymph glands) as a leishmanial form by binary fission (Fig. E, 1, 1*a*), later assuming the crithidial form (Fig. E, 2), which in its turn gives rise to the typical trypanosomes in the blood (Fig. E, 3). These may undergo equal binary fission (Fig. E, 3*a*, 3′), though, as in *Trypanosoma lewisi* and *Schizotrypanum cruzi*, such dividing forms are rarely encountered. It is probable, as suggested by Carpano, that the closely allied sheep trypanosome, *Trypanosoma melophagium*, develops in a similar manner.

The mode of reproduction of *Schizotrypanum pipistrelli* has more points of similarity with *Trypanosoma rabinowitschi* and *T. lewisi* than with *Schizotrypanum cruzi*, for its multiplication in the tissues of the bat takes place exclusively by binary fission in the crithidial stage (Fig. A, 2, 2*a*, 2′), from which the established trypanosome phase in the blood (Fig. A, 3) is derived. Morphologically the multiplicative phase of the bat trypanosome corresponds to the second type of reproduction in *Trypanosoma rabinowitschi* (cf. Fig. B, 2′, 2′*a*, 3).

From the account given above it is evident that the trypanosomes described have the following characteristic features in common. In the first place, in all of them multiplication in the vertebrate host is discontinuous, taking place only at definite periods which are succeeded by intervals during which the flagellates do not reproduce at all, or only occasionally. In the development of these trypanosomes there can thus be recognised a multiplicative and an established phase. In the second place, in all these forms reproduction during the multiplicative phase never takes place in the trypanosome stage, but in the crithidial and leishmanial stages.

As regards the remaining mammalian trypanosomes (belonging to Hoare and Coutelen's *evansi*, *vivax*, *congolense* and *brucei* groups), their development in the vertebrate differs from that of the trypanosomes just considered, in every respect. Their multiplication is not restricted to any special period, but is continuous, proceeding without interruption throughout the entire course of the infection. The typical method of reproduction in these species is by

equal binary fission in the trypanosome stage, the crithidial stage never occurring in the course of their development.

The different types of development among the mammalian trypanosomes are brought together in Table I and are illustrated in the figure.

Table I. *The development of trypanosomes in the mammalian host*

[The trypanosome groups correspond to those in the classification of Hoare and Coutelen (1933).]

Abbreviations and symbols

Bin. = binary. Div. = division. Eq. = equal. Mult. = multiple. Uneq. = unequal.

⊕ indicates the essential (characteristic) stages in the life-cycle.

\+ indicates unimportant stages, the significance of which in the life-cycle is not established.

Stages of development[1]		*lewisi* group[1]					Groups
		T. pipistrelli	*T. rabinowitschi*	*T. lewisi*	*T. cruzi*	*T. theileri*	*evansi vivax congolense brucei*
		A	B	C	D	E	F
Leishmanial:	1	.	.	.	⊕	+	+
Eq. bin. div.	1*a*	.	.	.	⊕	+	+
Crithidial:	2	⊕	⊕	⊕	⊕	⊕	.
Eq. bin. div.	2*a*	⊕	+	.	⊕	.	.
Eq. mult. div.	2*b*	.	⊕	.	.	.	.
Uneq. bin. div.	2*c*	.	.	+	.	.	.
Uneq. mult. div.	2*d*	.	.	⊕	.	.	.
Crithidial:	2′	⊕	⊕	⊕	⊕	.	.
Eq. bin. div.	2′*a*	.	⊕	⊕	.	.	.
Trypanosome:	3	⊕	⊕	⊕	⊕	⊕	⊕
Eq. bin. div.	3*a*	.	.	+	+	+	⊕
Trypanosome	3′	.	.	+	+	+	⊕

[1] The notation used in this table for the species and stages is the same as in the text-figure.

It will be seen that from the point of view of the method and morphology of reproduction, the mammalian trypanosomes fall into two distinct subdivisions, corresponding exactly to the two sections into which they are divided in Hoare and Coutelen's system of classification, namely: A, comprising the *lewisi* group, and B, comprising the groups of pathogenic trypanosomes. The data described in this paper thus introduce further important characters in the differential diagnosis of these two sections.

The various features described above are the characteristic and essential ones in the life histories of the trypanosomes in question. In addition to these, some trypanosomes assume stages which do not appear to be of much importance in their development, since they do not constitute necessary links in the life cycle, as in the case of the leishmanial forms of the African trypanosomes referred to above. However, the fact that such forms do occur provides further evidence of the affinity of these trypanosomes with the ones in which these stages represent an indispensable part of the developmental cycle.

Classification of the trypanosomes

We can now consider the bearing of the facts discussed above upon the classification of the mammalian trypanosomes, and especially upon their generic composition. In other words, we have to decide whether these

flagellates should be assigned to the single genus *Trypanosoma*, or whether some of them should be referred to the second genus, *Schizotrypanum*.

It has already been shown (cf. p. 101) that the fact that the representatives of the latter genus multiply in certain histological elements is not admissible as a systematic criterion, and is, moreover, not the exclusive characteristic of these flagellates. As regards the method of reproduction, it proceeds in the leishmanial stage only in one of them (*S. cruzi*), while the other (*S. pipistrelli*) reproduces in the crithidial stage. This and the fact that other trypanosomes are capable of multiplying in the leishmanial stage deprives this character of any generic value. As regards the other differential character of *Schizotrypanum*, viz. the absence of division in the trypanosome forms of blood, it has also been shown to be without foundation. It is thus evident that there is not a single morphological character which is the exclusive peculiarity of *S. cruzi*, or *S. pipistrelli*, and is not represented to a greater or less degree in the other trypanosomes.

On the other hand, it has been clearly demonstrated that the morphology of the trypanosomes, the stages of development, and method of reproduction of these two species in the vertebrate host are of the same type as in members of the *lewisi* group, and moreover there is a close similarity in the life cycle of these flagellates in the intermediate host.

As to the distinction between the trypanosomes under consideration, the structural difference between *S. cruzi* and *S. pipistrelli*, and between these species and any of the members of the *lewisi* group, is not greater than that which exists between the species of the last-named group themselves.

In view of all these facts, there remains no doubt that both *S. cruzi* and *S. pipistrelli* belong to the *lewisi* group of the genus *Trypanosoma*, as defined in the system of Hoare and Coutelen (1933), with the additional characteristics provided in the present paper. It is also obvious from what has been said that all these trypanosomes represent systematic units of the same category, and since they all conform to the generally recognised definition of the genus *Trypanosoma*, there is no need to raise any of them to a higher grade than species. If this were done, however, it would necessitate the creation of about as many genera (or subgenera) as there are species in the *lewisi* group and, consequently, to revise the definition of the genus *Trypanosoma*[1].

It has already been shown that there are a number of characters which separate the *lewisi* group as a whole from the other mammalian trypanosomes. Though also possessing all the attributes of the genus *Trypanosoma*, the latter have a number of important features in common which unite them into a section distinct from the *lewisi* group or section.

[1] I do not propose to discuss the question of the affinity between the trypanosomes and the leishmanias (cf. Dias' classification, p. 100). It will suffice to state that both morphologically and phylogenetically the two genera comprising these flagellates are disposed at the opposite poles of the family Trypanosomidae, and that the histotropism common to the leishmanias and some of the trypanosomes is no evidence of their relationship (cf. p. 101).

The mutual relations of the mammalian trypanosomes and the basis of their classification will be clear from Table II, which represents an amplification of the classification proposed previously (Hoare and Coutelen, 1933), especially as regards the *lewisi* group. It should be noted that this classification is based entirely on the morphology of the trypanosomes, the biological features adduced being only subsidiary characteristics of the sections. It is noteworthy that the two sections are opposed to each other almost in every single character, morphological as well as biological.

I hope to deal more extensively with the trypanosomes of section B in a future paper. In the meantime, the characteristics of the various groups belonging to that section will be found in the earlier publication on this subject (Hoare and Coutelen, 1933).

Table II. *Classification of the mammalian trypanosomes*

Fam. TRYPANOSOMIDAE

Gen. **Trypanosoma**

Definition: Flagellates which, in the course of their development in the vertebrate and invertebrate hosts, pass through the leishmanial, leptomonad, crithidial and trypanosome stages, attaining the highest degree of morphological complexity within the family.

SECTION A

Morphology of trypanosomes in vertebrate host:
Kinetonucleus not terminal. Posterior end of the body drawn out and pointed. Division typically in the crithidial and leishmanial stages (exceptionally in the trypanosome stage).

Biological characteristics:
Multiplication discontinuous in the vertebrate host. Cyclical development in the intermediate host; infective forms (metacyclic trypanosomes) produced in hind-gut (posterior station). Transmission contaminative (through faeces). Trypanosomes slightly or not pathogenic; easily culturable.

Lewisi group:
Reproduction by equal binary fission in the crithidial stage: **T. pipistrelli** (=**T. vespertilionis**?)
Reproduction primarily by equal multiple fission and secondarily by binary fission in the crithidial stage: **T. rabinowitschi** (=**T. criceti**)
Reproduction primarily by unequal multiple fission and secondarily by binary fission in the crithidial stage (occasionally division in the trypanosome stage): **T. lewisi, T. duttoni, T. primatum**?
Reproduction primarily by binary fission in the leishmanial stage and secondarily in the crithidial stage (occasionally division in the trypanosome stage): **T. cruzi, T. theileri**?
Trypanosomes the method of reproduction of which is insufficiently known: **T. melophagium, T. theodori** (and various other trypanosomes of rodents, insectivores, carnivores, etc.).

SECTION B

Morphology of trypanosomes in vertebrate host:
Kinetonucleus terminal or sub-terminal. Posterior end of the body blunt. Division typically in the trypanosome stage.

Biological characteristics:
Multiplication continuous in the vertebrate host. Cyclical development in the intermediate host; infective forms (metacyclic trypanosomes) produced in the proboscis or salivary glands (anterior station). [Except in the *evansi* group in which there is no cyclical development.]
Transmission inoculative (through the bite). [Except in *T. equiperdum*.]
Trypanosomes pathogenic, not easily culturable.

Evansi group: **T. evansi, T. equinum, T. equiperdum**, etc.

Vivax group: **T. vivax, T. caprae, T. uniforme.**

Congolense group: **T. congolense, T. simiae.**

Brucei group: **T. brucei, T. rhodesiense, T. gambiense.**

SUMMARY

This paper is devoted to a critical analysis and revision of the systematics of the mammalian trypanosomes. It is shown that there is no justification for the recognition of more than one genus, *Trypanosoma*.

The trypanosomes are subdivided into two sections, each with distinct morphological and biological characteristics.

In the section corresponding to the *lewisi* group the method of reproduction is used as a criterion for the specific differentiation of the trypanosomes.

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THE SEASONAL OCCURRENCE OF HARVEST MITES (*TROMBICULA AUTUMNALIS* SHAW) ON VOLES AND MICE NEAR OXFORD

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(With 1 Figure in the Text)

I. Introduction

In the course of an investigation of the parasites of rodents in Bagley Wood near Oxford during the years 1925–8 (Elton, Ford, Baker and Gardner, 1931), a large collection of *Trombicula* larvae was made, but not reported upon fully at the time because the specimens had not all been examined. Since then, one of us (G. K.) has made a study of the collection which enables the statistical data to be published. It has not been possible to identify every specimen, on account of variations in the state of preservation and the loss of some material; but it is believed that the sample is sufficiently large to prove that only one species (the same that attacks human beings) was present. The specimens agree in essential points with the figures given by Hirst (1915) and André (1930) and have been compared with British Museum material, for access to which we have to thank Dr Susan Finnegan. Further confirmation is provided by the earlier reports (noted in the paper above) from Graf von Vitzthum and Dr Louis Sambon, both of whom examined a certain number of specimens. Only one species of *Trombicula* has been found attacking man or other mammals in Europe and the British Isles, although two other species of the genus (*T. canestrinii* and *T. formicarium*) are known from the Continent.

II. Results

Harvest-mite larvae were found on three species of rodents: the wood-mouse (*Apodemus sylvaticus* L.), the bank vole (*Clethrionomys* (=*Evotomys*) *glareolus britannicus* (Miller)), and the field vole (*Microtus agrestis hirtus* (Bellamy)). Although a large number of shrews (mainly *Sorex araneus castaneus* Jenyns, but also some *S. minutus* L.) were examined, *Trombicula* larvae were never found on them. This is interesting, considering that they do occur on such a large number of small mammals and birds, and that shrews use the runs of voles and mice. André (1930) records them from the water shrew *Neomys fodiens*. The *Apodemus* and *Clethrionomys* were trapped in mature woods and plantations of Bagley Wood, partly in conifer and partly in oak. The *Microtus* were mainly trapped on open sandy grassland on Boar's Hill, also a few in fields near Wolvercote. Each mouse or vole was searched very thoroughly all over for parasites, all of which were preserved.

The position of the parasite varies in different hosts. On *Clethrionomys* the most usual habitat of *Trombicula autumnalis* is inside the ears. 100 per cent. had harvest mites in their ears and 5 per cent. had them also round the anus or on the genital organs. These exceptions occurred in August and September. On *Apodemus* the characteristic habitat is round the anus and on the genital organs. This was the habitat in 89 per cent. of the infested specimens. Of these 9 per cent. were also parasitised on the ears, body and legs. 11 per cent. were parasitised only in these places. On *Microtus*, the mites are found almost invariably in the ears alone. 100 per cent. had the mites in their ears and only 4 per cent. (one record) had them also on the body. The exception occurred in August.

Table I. *Monthly occurrence of* Trombicula autumnalis *on voles and mice*

	Apodemus sylvaticus			*Clethrionomys glareolus*			*Microtus hirtus*		
Date 1926	No. of mice examined	No. with *Trombicula*	% with *Trombicula*	No. of voles examined	No. with *Trombicula*	% with *Trombicula*	No. of voles examined	No. with *Trombicula*	% with *Trombicula*
Aug.	19	8	**42**	14	11	**79**	9	1	**11**
Sept.	62	25	**40**	31	28	**90**	6	4	**67**
Oct.	67	31	**46**	28	22	**79**	26	6	**23**
Nov.	54	7	**13**	34	21	**62**	31	0	**0**
Dec.	35	1	**3**	22	9	**41**	30	0	**0**
1927									
Jan.	40	0	**0**	6	2	**33**	35	0	**0**
Feb.	39	0	**0**	3	1	**33**	32	0	**0**
Mar.	38	0	**0**	31	8	**26**	24	0	**0**
Apr.	35	0	**0**	28	7	**25**	24	0	**0**
May	21	0	**0**	23	2	**9**	22	0	**0**
June	34	0	**0**	31	0	**0**	30	0	**0**
July	31	2	**6**	14	4	**29**	22	1	**5**
Aug.	31	6	**19**	21	18	**86**	24	8	**33**
Sept.	32	24	**75**	30	28	**93**	—	—	—
Oct.	35	11	**31**	12	11	**92**	13	2	**15**
Nov.	91	6	**7**	16	12	**75**	19	1	**5**
Dec.	51	0	**0**	16	9	**56**	—	—	—
1928									
Jan.	80	0	**0**	21	9	**43**	—	—	—
Feb.	38	0	**0**	22	7	**32**	—	—	—
Mar.	37	0	**0**	3	0	**0**	—	—	—
Apr.	42	0	**0**	15	4	**27**	—	—	—
July	—	—	—	12	7	**58**	—	—	—

Table II. *Quarterly occurrence of* Trombicula autumnalis *on voles and mice. Percentage with* Trombicula

Date		*Apodemus sylvaticus*	*Clethrionomys glareolus*	*Microtus hirtus*
1926.	Aug.–Oct.	**43**	**84**	**27**
1926–7.	Nov.–Jan.	**6**	**52**	**0**
1927.	Feb.–Apr.	**0**	**26**	**0**
1927.	May–July	**2**	**9**	**1**
1927.	Aug.–Oct.	**42**	**90**	**27**
1927–8.	Nov.–Jan.	**3**	**57**	—
1928.	Feb.–Apr.	**0**	**28**	—

Tables I and II and Fig. 1 show the seasonal occurrence of *Trombicula*. The detailed figures are deposited in the Bureau of Animal Population at Oxford. The quarterly figures in Table II give a more reliable picture statistically than the monthly figures in Table I. The percentage on *Clethrionomys* in March 1928 has been put as a query in the figure, since the absence of larvae must clearly have been due to the very small numbers of voles examined that month. There is a sudden appearance of larvae at the end of July. (Earliest dates (1927): *Apodemus*, July 28th; *Clethrionomys*, July 28th;

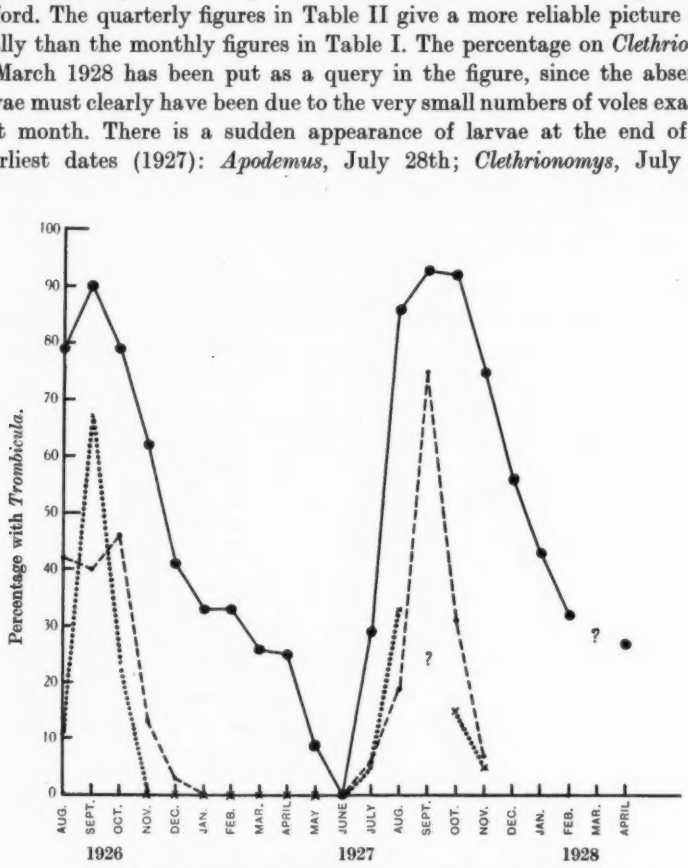

Fig. 1. Percentage of mice and voles with *Trombicula autumnalis*.
--- *Apodemus sylvaticus*. • • • *Microtis agrestis hirtus*. —— *Clethrionomys glareolus*.

Microtus, July 20th.) The highest rate of infestation occurs in September, after which the numbers decline throughout the early winter. (Latest dates: *Apodemus*, December 1st, 1926, November 16th, 1927; *Microtus*, October 30th, 1926, November 14th, 1927.) On *Clethrionomys* mites were found throughout both winters, until half way through May (latest dates: May 14th, 1927, April 14th, 1928). It should be noted that although monthly quotas were usually spread out over a good many days of the month, there is room for a possible variation of a week or two before or after the dates mentioned, owing to mice not being trapped, or the quotas being small.

The disappearance of larvae from *Apodemus* might be accounted for by the external attachment of the mites, which would be more easily killed or knocked off. But this does not explain the very similar curve of infestation found in *Microtus* where the larvae come in the ears. Therefore the factor determining successful winter survival on a host cannot be simply protection or warmth. The figures prove that the bank vole can act as an effective winter reservoir of larvae, and may therefore be locally of importance in maintaining the harvest-mite population, and that *Apodemus* and *Microtus* were not of importance in this respect near Oxford in 1926–8. One of us (G. K., unpublished) has found that the rabbit (*Oryctolagus cuniculus* (L.)) also acts as a winter host in some places near Oxford.

The adult of *Trombicula* has not yet been found in England, an astonishing fact which Warburton (1928) has discussed. Since his paper appeared, a unique specimen has been found in France by André (1929): this he believes to be the adult. The enormous abundance of larvae that infest some parts of the country every summer, the rarity of adults, and the association with rodents, has suggested (Hirst, 1926) that the later stages might be found in rodent nests. André believes, however, that they live on the ground and in soil. The seasonal curve for *Clethrionomys* makes it likely that the adults should be looked for in June and July—the period between the last occurrence of *Clethrionomys* and the first outburst of new larvae. On the other hand, it has to be remembered that the percentage of old voles in the population falls rapidly during the spring months, owing to deaths from old age and increase in numbers of young, so that the examination of a fixed quota each month may give a misleading drop in parasite infection. The numbers caught each month are not large enough to permit separate analysis of old and young voles.

SUMMARY

1. Examination of monthly quotas of *Apodemus sylvaticus*, *Clethrionomys glareolus*, and *Microtus hirtus*, for parasites over a period of 21 months, enabled the seasonal occurrence of *Trombicula autumnalis* larvae to be determined.

2. All three species are infested during the summer and autumn months, and *Clethrionomys* throughout the following winter and spring also. Shrews were not parasitised.

3. The mites occupy different positions on different hosts.

4. *Clethrionomys* and rabbits (*Oryctolagus*) are of importance in maintaining the stock of harvest mites on some areas in the south of England.

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THE BIOLOGY OF AUTOGENOUS AND ANAUTOGENOUS RACES OF *CULEX PIPIENS* L. (DIPTERA: CULICIDAE)[1]

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(With 5 Figures in the Text)

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I. Introduction

Roubaud and his co-workers have shown that in France there are definite biological races of *Culex pipiens* L. (Roubaud, 1929, 1930, 1930*a*, 1931, 1931*a*, 1933; Roubaud and Toumanoff, 1930, 1930*a*; and Roubaud and Gaschen, 1932).

[1] This work was done on behalf of the Chemotherapy Committee of the Medical Research Council, London, in connexion with work on the biology of avian malaria.

Two French races are distinguished by these authors: (1) *C. pipiens pipiens* which undergoes cyclical hibernation (asthenobiosis), does not oviposit without blood meals (anautogeny), and requires a large space for pairing (eurygamy). (2) *C. pipiens autogenicus* which is a non-hibernating race that breeds continuously throughout the year in cesspits, drains, cellars and similar habitats. Females of this race lay fertile eggs without any food in the imaginal stage, the ova being matured at the expense of reserves accumulated during larval life (autogeny); and fertilisation occurs even in very small vessels (stenogamy).

De Boissezon (1929, 1929*a*, 1930, 1930*a*, 1934) found autogeny in *C. pipiens* in the south of France but ascribes it to the conditions of life, especially as regards the larval food, a diet rich in iron favouring maturation of the ova and resulting in autogeny. This author denies the occurrence of cyclical hibernation (asthenobiosis) in *C. pipiens* and considers that hibernation is due to a general slowing down of metabolism caused by a decrease in temperature. Hecht (1932) and Weyer (1934, 1934*a*) have described autogeny in German *C. pipiens*; and Hecht considers that hibernation is not cyclical but is induced by low temperature. La Face (1926) and Nieschulz and Bos (1931) found that overwintering females could be easily reactivated and concluded that hibernation is not due to cyclical asthenobiosis. De Boissezon, Hecht, Weyer, and Nieschulz and Bos in their work seem to have concentrated attention on the points of cyclical hibernation and oviposition without blood meals and did not take into consideration the most constant characteristic of Roubaud's autogenous race which is stenogamy.

MacGregor (1932) described an autogenous race of *C. pipiens* from Germany and found that it bred true for a number of generations and differed greatly in behaviour from an English anautogenous hibernating race. Recently Marshall and Staley (1935) have found an autogenous race of *C. pipiens* in the South of England. Vincent (1933) and Tate and Vincent (1934) recorded autogenous races of *C. pipiens* from Hungary, Malta and Greece.

Other workers (Grassi, 1923; Legendre, 1931, 1931*a*, 1932) have noted the occurrence of races of *C. pipiens* but have not demonstrated the biological differences recorded by Roubaud and his co-workers. Previous to Roubaud oviposition without a blood meal was recorded in *C. pipiens* by Neumann (1912), Huff (1929) and others, but these authors did not distinguish a definite race of *C. pipiens* which does not require blood meals for oviposition. Recently Roubaud (1935) has described morphological differences between the egg floats of the two races.

For the past three years we have maintained three strains of autogenous *C. pipiens* (from Hungary, Greece, and Malta) in the laboratory and have studied them comparatively with several strains of English hibernating, anautogenous, *C. pipiens*. The main results of this comparative study of the two races are given in the present paper.

II. THE ENGLISH ANAUTOGENOUS RACE OF *CULEX PIPIENS*

(1) *Reactivation of hibernating females by artificial feeding*

As this work was begun in the winter, in order to obtain cultures, the reactivation of hibernating females was attempted by artificially feeding them on blood by MacGregor's (1930) capillary tube, unsheathed proboscis, method. MacGregor (1931) found that when fed on blood in this manner females of the spring generation oviposited, but that females of the winter, hibernating, generation did not oviposit even after several feedings. We tried

Table I. *Oviposition by anautogenous English* Culex pipiens *after artificial feeding and natural gorging*

Females	Kind of blood	Month	1st feeding no. laid/ no. fed	2nd feeding no. laid/ no. fed	3rd feeding no. laid/ no. fed	Total no. laid/ no. fed
		Artificial feedings				
Hibernating	Canary, citrate	Dec.	2/13	4/13	1/7	7/13
,,	,,	Jan.	1/40	5/23	—	6/40
,,	Canary, haemolysed	Jan.	0/12	—	—	0/12
,,	Fowl, citrate	Jan.	3/10	—	—	3/10
,,	Toad, citrate	Jan.	1/1	—	—	1/1
,,	Human, citrate	Dec.	0/7	0/7	—	0/7
,,	,,	Jan.	0/20	0/6	—	0/20
,,	Ox, whole	Jan.	2/11	—	—	2/11
Laboratory bred	Ox corpuscles in saline	Jan.	0/10	—	—	0/10
,,	,,	Feb.	0/28	0/19	0/19	0/28
Hibernating	Ox serum	Jan.	0/9	—	—	0/9
		Natural gorging				
,,	Canary	Jan.	6/7	—	—	6/7
,,	,,	Feb.	16/20	—	—	16/20
,,	,,	Apr.	6/8	—	—	6/8
Laboratory bred	Human	Jan.	2/4	—	—	2/4
Hibernating	,,	Jan.	3/6	—	—	3/6
			5/10	—	—	5/10

feeding females on different kinds of blood and on different fractions of blood (serum alone, washed corpuscles alone), and the results of these experiments, which are given in detail in Table I, may be stated briefly as follows.

Fifty-three females were fed once on *citrated canary blood* and three of them laid eggs. Thirty-six of them were fed a second time and nine laid egg rafts. Seven were fed a third time and one laid a raft. In all, of the fifty-three females fed once or more, fourteen laid egg rafts, that is, about 26 per cent. of those fed.

No eggs were obtained from twelve females fed once on *haemolysed canary blood*. Of ten females fed once on *citrated fowl blood* three laid rafts; and one female fed once on *citrated toad blood* laid a raft.

It is interesting that of twenty-seven females fed on *citrated human blood* none laid eggs, although thirteen of them were fed a second time. Two females, out of eleven, fed once on ***whole ox blood*** laid rafts; but no rafts were laid by twenty-eight females fed once on ***ox corpuscles in saline***, or by nine females fed on *ox serum*.

During the same period thirty-five females gorged naturally on canaries, and twenty-eight of them, or 80 per cent., laid egg rafts; while of ten females which gorged on man five laid rafts.

All of the experiments, except one natural gorging experiment, were done in December, January and February, and it is, therefore, clear that English hibernating *C. pipiens* females can be reactivated during the cold months by artificial feeding on blood; and that those which are induced to gorge naturally lay readily after a single blood meal and do not show gonotrophic dissociation as described by Roubaud (1933). The fact that eggs were not obtained after artificial feeding with human blood supports the conclusion of Roubaud and Mezger (1934) that bird blood is more favourable than human blood for the maturation of ova in *C. pipiens* females. It is possible that the negative results recorded by MacGregor (1931) for hibernating females were due to his having used human blood, but the kind of blood used is not stated in his paper.

The experiments also indicate that amphibian blood as well as bird blood is suitable for maturation of ova in *C. pipiens*; and that, at least in the case of ox blood, the whole blood is necessary and neither the serum alone nor the corpuscles alone is sufficient for the development of eggs.

(2) *Influence of light upon the gorging of females of the anautogenous race of* Culex pipiens

When adults were raised from the eggs obtained from the artificially fed females, at first difficulty was experienced in inducing the resulting females to gorge. The difficulty of inducing females to gorge was encountered by previous workers including Sister Monica Taylor (1914, 1917), and MacGregor and Chung Un Lee (1929). We found, however, that prolonged exposure to light (daylight or artificial) caused a large proportion of the females to gorge when they were subsequently placed with birds in the dark; whereas only very few females would gorge if they were kept in the dark or in dim light continuously (Tate and Vincent, 1932).[1] Further experiments have been made with many different lots of *C. pipiens*, and the results confirm our preliminary work as to the influence of illumination on gorging.

[1] Some other workers have misinterpreted our note on the influence of light on the gorging of females and think that we said that *C. pipiens* females bite better in the dark than in light. Hence we wish to emphasise that what we stated is that if females are *first illuminated for a long period* and are *then placed in the dark* with birds a much higher percentage of them will gorge than if they had not been illuminated previously.

The experiments were mostly done as follows:

A glass-walled cage was divided by a central partition into two similar compartments, each of which could be completely darkened by means of sliding screens. In each compartment were placed a small bird cage, a wide-mouthed bottle of water into which dipped a fan of absorbent paper to preserve a high humidity, a thermometer, and, except in some experiments during which the females were starved, a Petri dish containing a slice of apple or raisins. During the experiments one compartment was darkened by means of the screens whilst the other was illuminated by daylight, or by an electric bulb, shining through a water trough to prevent heating the illuminated compartment of the cage. Temperatures were recorded daily and did not vary by more than 0·5° C. between the two compartments. In some experiments paper hygrometers were placed in each compartment, but there was no significant difference in the relative humidity (about 70–80 per cent.) of the two compartments.

The conditions of the experiments were varied as regards temperature; presence or absence of food; nature of illumination, daylight, artificial light or both; and period of access to birds for gorging; but as the final result is that illuminated females gorge more readily than those kept in darkness, it is unnecessary to describe the experiments in detail and only a selection of those done is tabulated in Table II. The results are shown graphically in Fig. 1.

The most interesting fact which emerges from these experiments is that hibernating females of English anautogenous *C. pipiens*, collected during September, October and November, gorged readily after continuous illumination for about 10 days both at room temperature and at 25° C. 70–80 per cent. of the illuminated females gorged, while of the control females kept in the dark only 3–13 per cent. gorged (Exps. 1, 2, 3, Table II and Fig. 1). That it is not simply a question of starvation of the illuminated females rendering them more voracious is proved by the fact that in Exp. 3 they were given no food in either the dark or illuminated compartments during the period of the experiment, and nevertheless only 3 per cent. of those in the dark gorged compared with 71 per cent. of those which had been illuminated.

At room temperature similar results were obtained with females bred in the laboratory whether they were illuminated solely by artificial light, as in Exp. 4, or by daylight and additional artificial light at night, as in Exp. 8. In some experiments, as in No. 4, females were also exposed to natural daylight without additional illumination, and the percentage of those which gorged was intermediate between those continuously illuminated and those kept in darkness. Usually 60–70 per cent. of the illuminated females gorged, 10 per cent. or less of those exposed to winter daylight alone, and only 3–6 per cent. or less of those kept in darkness (Table II, Fig. 1).

At 25° C. the results obtained with laboratory-bred females were more irregular. In most cases the percentage of illuminated females which gorged (56–74 per cent.) was much greater than the percentage of those kept in darkness (13–29 per cent.), but, as is shown by Exps. 9 and 10, a relatively higher percentage of females kept in the dark at 25° C. gorged than of similar females kept at room temperature. In one experiment, No. 7, done at 22° C., 69 per cent. of the females kept in the dark, and 98 per cent. of those illumi-

Table II. *Influence of light upon the gorging of the English anautogenous race of* Culex pipiens

Exp.	Kind of females and time of year	Treatment	Temp. °C.	Food	Interval before gorging began days	Period of gorging hours per day	Deaths during exp.	No. gorged/no. survived = % gorged
1	English hibernating, September	(*a*) Dark	Room	Apple	8	3 × 14	9	2/78 = 2·5 %
		(*b*) Day + art.	,,	,,	8	3 × 14	7	57/77 = 74 %
2	English hibernating, October	(*a*) Dark	,,	,,	6	3 × 9	16	6/47 = 13 %
		(*b*) Art. con.	,,	,,	6	3 × 9	8	45/51 = 88 %
3	English hibernating, November	(*a*) Dark	25	None	10	3½ × 1	2	1/38 = 3 %
		(*b*) Art. con.	25	,,	10	3½ × 1	2	27/38 = 71 %
4	English laboratory bred, August	(*a*) Dark	Room	Apple	8	3 × 8	—	0/80
		(*b*) Art. con.	,,	,,	8	3 × 8	—	59/80 = 74 %
		(*c*) Daylight	,,	,,	8	3 × 8	—	3/80 = 4 %
5	English laboratory bred, November	(*a*) Dark	,,	,,	8	3 × 4	—	1/80 = 1 %
		(*b*) Art. con.	,,	,,	8	3 × 4	—	32/80 = 40 %
		(*c*) Daylight	,,	,,	8	3 × 4	—	3/80 = 4 %
6	English laboratory bred, October	(*a*) Dark	,,	,,	8	5 × 1	—	3/50 = 6 %
		(*b*) Art. con.	,,	,,	8	5 × 1	—	29/51 = 58 %
7	English laboratory bred, June	(*a*) Dark	22	,,	6	3½ × 1	1	68/99 = 69 %
		(*b*) Art. con.	22	,,	6	3½ × 1	4	94/95 = 98 %
8	English laboratory bred, September	(*a*) Dark	20	,,	4	2½ × 1	—	1/80 = 1 %
		(*b*) Day + art.	20	,,	4	2½ × 1	—	59/79 = 75 %
9	English laboratory bred, November	(*a*) Dark	25	,,	6	1½ × 1	3	6/47 = 13 %
		(*b*) Art. con.	25	,,	6	1½ × 1	11	22/39 = 56 %
10	English laboratory bred, January	(*a*) Dark	25	,,	11	3½ × 1	4	9/35 = 29 %
		(*b*) Art. con.	25	,,	11	3½ × 1	—	25/38 = 66 %
11	English laboratory bred, December, in large cages	(*a*) Daylight	Room	,,	17	2½ × 1	—	0/170
		(*b*) Day + art.	,,	,,	17	2½ × 1	—	71/158 = 45 %
12	English laboratory bred, gorged, August	(*a*) Dark	,,	,,	13	3 × 6	13	9/24 = 38 %
		(*b*) Daylight	,,	,,	13	3 × 6	26	9/21 = 43 %
13	English laboratory bred, gorged, March	(*a*) Dark	,,	Raisin	22	22 × 1	—	1/15 = 9 %
		(*b*) Art. con.	,,	,,	22	22 × 1	—	0/16
14	English laboratory bred, gorged, January	(*a*) Dark	25	Apple	13	3 × 1	2	13/27 = 48 %
		(*b*) Art. con.	25	,,	13	3 × 1	5	2/25 = 8 %
15	Greek autogenous, September	(*a*) Dark	Room	,,	22	1 × 1	—	20/29 = 69 %
		(*b*) Day + art.	,,	,,	22	1 × 1	—	25/31 = 83 %
16	Greek autogenous, November	(*a*) Dark	,,	,,	5	4½ × 1	8	30/43 = 70 %
		(*b*) Art. con.	,,	,,	5	4½ × 1	3	39/45 = 87 %
17	Hungarian autogenous, November	(*a*) Dark	,,	,,	6	3 × 8	—	22/70 = 31 %
		(*b*) Art. con.	,,	,,	6	3 × 8	—	29/70 = 41 %
18	Maltese autogenous, August	(*a*) Dark	,,	,,	6	2 × 1	6	22/53 = 42 %
		(*b*) Art. con.	,,	,,	6	2 × 1	0	24/59 = 40 %

Explanation of Table II. In column 3, Treatment, "Dark" means that the females were kept in continuous darkness; "Daylight" means that they were exposed to natural daylight; "Day + art.", that they were exposed to natural daylight and in addition were illuminated artificially at night; "Art. con.", that they were exposed to continuous illumination with artificial light during the experiment, except while birds were put in for the mosquitoes to gorge upon. In column 4, "Room" temperature means that the experiment was done at ordinary laboratory temperature which varied between 17 to 21° C.; where the temperature is given specifically, the experiments were done in constant temperature rooms. In column 6 the numbers given are the number of days for which the experiment was run before birds were put in to be bitten. In column 7, 3 × 14 means that birds were put in for about 3 hours a day over a period of 14 days; similarly, 3½ × 1 means that all the gorging took place in 3½ hours on 1 day. The number of deaths recorded in column 8 is important, as it shows that continuous illumination is not unfavourable to the lives of the females over the times of the experiments. In the last column the figures represent, in each case, the number of females which gorged out of the number of females surviving at the end of the experiment and the corresponding percentages for these figures.

nated, gorged. This experiment was quite exceptional for the high percentage of gorging under both conditions.

The matter can be summed up by saying that at temperatures up to about 20° C. illumination for 5–10 days induces an average of about 70 per cent. of females of the English anautogenous race of *C. pipiens* to gorge, whereas only 6 per cent. or less of those kept in the dark will gorge. At 25° C. a large percentage of females kept in the dark may sometimes gorge but cannot be relied upon to do so; but 55–70 per cent. of the illuminated females will gorge regularly. We have never had a case in which sufficiently illuminated females failed to gorge in a large proportion when given an opportunity of doing so.

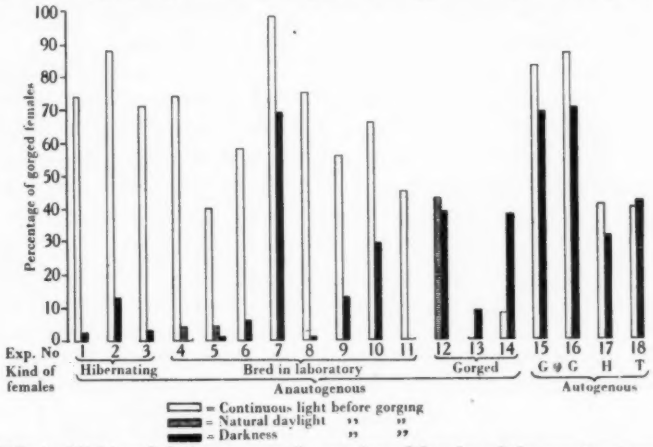

Fig. 1. Effect of light and darkness upon the gorging of females of the anautogenous race of *C. pipiens*.

Similar experiments were done with gorged females to see if light influences the second gorging, but the results were negative and in some cases a higher percentage of those kept in the dark gorged. The figures for three experiments (Table II; Fig. 1) are: No. 12, light 43 per cent., dark 38 per cent., No. 13, light 0 per cent., dark 9 per cent., No. 14, light 8 per cent., dark 48 per cent.[1]

Autogenous races of Greek, Maltese and Hungarian *C. pipiens* were also tested, and it was found that light does not significantly influence the gorging of these mosquitoes (Exps. 15–18, Table II; Fig. 1). Thus, for the Greek females the percentages of gorged females were: Exp. 15, light 83 per cent., dark 69 per cent.; Exp. 16, light 87 per cent., dark 70 per cent.; for the Hungarian females: Exp. 17, light 41 per cent., dark 31 per cent.; and for the Maltese females: Exp. 18, light 40 per cent., dark 42 per cent.[1]

[1] In the case of gorged anautogenous females and of autogenous females when they were illuminated for the above experiments, although in the tabulated material the term "continuous" illumination is used for brevity, in fact such females were kept dark for a short time each day to give them an opportunity of ovipositing. Strictly speaking, these females were illuminated for 22–23 hours a day and not continuously. Under these conditions, oviposition took place readily in both the darkened and illuminated compartments.

The mechanism of the effect of light on the anautogenous females is still obscure. In our preliminary note (1932) we mentioned that illumination seems to stimulate development of ovaries and to hasten disappearance of fat body. Further experiments have shown that, while true of many cases, such effects are irregular. In some experiments there may be marked differences between the size of the ovaries of the "dark" and "light" females, but in other experiments the differences are slight, and individuals of an illuminated lot may retain large fat-body while some females kept in the dark may show large ovaries. With hibernating females the illumination definitely appears to hasten the absorption of fat-body, and after a long period of illumination, 10 days or more, most of the illuminated females are obviously much thinner than the control females kept under similar conditions in darkness.

As Exps. 1, 2 and 3 (Table II, Fig. 1) clearly demonstrate, prolonged illumination of females collected in a state of hibernation is very effective in reactivating them. Over 70 per cent. of the illuminated females become active and gorge, compared with 13 per cent. or less of those kept in the dark, and the reactivated females readily lay egg rafts after one blood meal. The latter point will be discussed later in relation to gonotrophic dissociation.

The effect of illumination is of importance in relation to the breeding of the anautogenous race of *C. pipiens* as, in our experience, under natural conditions of illumination, it is not possible to breed this race continuously throughout the year. In the dark winter months the females become sluggish, develop fat-body, and refuse to gorge.[1] If, however, they are illuminated artificially at night they remain active and can be kept breeding continuously throughout the year. The following experiment illustrates the activating influence of additional artificial illumination during the winter.

Exp. 11 (Table II, Fig. 1). Several hundred pupae bred in December were placed in large cages (60 cm. square) to emerge. One cage (A) was left in natural daylight; whilst the other (B) was illuminated at night by a 60-watt electric bulb. After 17 days a canary was put into each of the cages, which were darkened, for 2½ hours. Of the 170 females in cage A (natural light) not one gorged; but of the 158 females in cage B (artificial light at night), 71 (=45 per cent.) gorged.

(3) *Continuous breeding of anautogenous* Culex pipiens *in the laboratory*

By utilising the effect of illumination during the dark winter months we have kept the anautogenous race of English *C. pipiens* breeding throughout the year in the laboratory. This strain was begun in March 1934 with a single fertile raft laid by F_1 progeny of hibernating females collected in October 1933. The strain has been breeding continuously since and has passed through eleven generations. From each generation a number of females was put aside in a cool basement in a moist atmosphere, without food, and left until they died to determine if hibernation was a cyclical phenomenon in this strain and

[1] Recently Jobling (1935) has found that under ordinary laboratory conditions English anautogenous *C. pipiens* cease breeding during the winter.

if they would undergo "autotrophic" fat-body development as described by Roubaud (1931*a*, 1933). Before considering the results of these experiments, which are briefly summarised in Table III, a short description of the methods used may be given:

The larvae were kept in tap water in enamelled dishes 18 cm. in diameter by 5 cm. deep, and were fed by sprinkling food on the surface of the water daily. The food used was dried and finely powdered brown bread (MacGregor, 1932), finely ground lentils (de Boissezon, 1934), ground dog biscuit, or boiled yeast. The water was changed from time to time when it became too foul. The larvae were reared in a thermostat at 24° C. Pupae were transferred to clean water and were placed in a large cage, 75 cm. square, to emerge. The large cage was kept at room temperature, 17–20° C., and contained dishes of water and wet lint to preserve a moist atmosphere, about 80 per cent. relative humidity, and slices of apple. When females required blood meals canaries were put in, and the cage darkened, for a few hours daily until a sufficient number had gorged. The cage could be illuminated when necessary either from within or from the outside by a 60-watt electric light connected to an automatic switch set to turn on the light about 12 p.m.

Experiments to test for "autotrophic" fat-body development and also determine the survival rates of females starved and fed on fruit (see later) were arranged as follows:

An equal number of females was placed in each of two glass cylinders, 12 cm. high by 9·5 cm. wide, covered on top and bottom with cotton gauze. The cylinders contained absorbent paper for the females to rest upon, and were suspended side by side in a large glass trough containing a layer of water at the bottom, so that the gauze-covered bottoms of the cylinders were about 2 cm. above the surface of the water. A pad of wet cotton lint was placed on the top gauze of each cylinder, and, in addition, a slice of apple was placed on top of one cylinder. Finally, the top of the trough was covered with a plate of glass so that a saturated atmosphere was maintained within. Thus the two lots of females were kept in a saturated atmosphere under similar conditions except that those in one cylinder were starved and those in the other were fed on apple. The fed females were given apple for about 5 weeks and, as during this time they mostly became very fat, the apple was then removed and they were subsequently starved until they died to test how long such fat females could survive in the absence of any food.

When in each generation a sufficient number of females had gorged in the large cage, they were removed and placed in cardboard cylinders standing over dishes of water in a thermostat at 24° C., as it was found that they laid more readily in a small container in close contact with water, a fact previously noted by Legendre (1928). An interesting point is that a much larger proportion of the rafts laid the first few days was fertile than of those laid later. Possibly the inseminated females mature eggs more rapidly and oviposit sooner after a blood meal than do unpaired females. In the winter months, although the cage had glass sides and top and was exposed to natural daylight in a well-lighted room, the females would rarely gorge when birds were put in before the cage had been illuminated at night. After 4 or 5 nights' illumination, however, the females began to gorge voraciously.

It will be seen from Table III that under these conditions, in this strain of *C. pipiens*, only a proportion of the rafts laid was fertile. In our experience,

a cage about 60 cm. square is the smallest area in which fertilisation takes place regularly with the anautogenous, eurygamous race, although occasionally a fertile raft may be obtained from mosquitoes kept in smaller cages. The percentage of fertile rafts varied in different generations from 14 to 54 per cent. with an average for the eleven generations of 33 per cent. It is notable that the highest percentage of fertile rafts was laid in the winter months, September to December, when hibernation should occur if it were a cyclical phenomenon in this race. It may be mentioned that the percentage of fertile rafts from three other strains of the English anautogenous race which were bred in the laboratory for a number of generations was 26 per cent. (90 hatched out of 340); 44 per cent. (195 hatched out of 441); and 53 per cent. (161 hatched out of 305).

Table III. *Continuous breeding of the English anautogenous race of* Culex pipiens *for eleven generations in the laboratory. The table shows the percentage of fertile rafts obtained throughout the eleven generations and the survival times of unfed females*

					Survival of unfed females			
Generation	Month	No. rafts laid	No. rafts hatched	% of rafts fertile	Temp. °C.	No. of females	Average survival in days	Maximum survival in days
F_1	Mar.	6	1	17	—	—	—	—
F_2	May	59	8	14	16–19	19	10·8	13
F_3	June	154	26	17	17–20	46	7·5	11
F_4	July	308	91	30	—	49	—	9
F_5	Aug.–Sept.	256	67	30	17–20	29	10·5	19
F_6	Sept.–Oct.	209	95	45	18–19	16	8·7	15
F_7	Dec.	145	78	54	16–17	16	6·2	8
F_8	Jan.	204	57	28	15–17	18	11·3	19
F_9	Feb.	421	144	34	12–15	15	13·3	15
F_{10}	Mar.	239	92	38	14–17	25	10·9	17
F_{11}	April	144	49	34	14–16	21	10·0	12
F_1 to F_{11}		2145	708	33	—	254	—	19

(4) *Survival of fed and unfed females*

It can be seen from Table III that the average length of life of unfed females, that is, females which were given neither fruit nor blood, varied from one generation to another in an irregular manner. The average length of life in the F_7 generation was only 6·2 days, while in the F_9 generation it was 13·3 days. Although during these experiments the temperature fluctuated considerably, it appears that the average length of life of the females of the different generations was not decided by this factor. Thus the average for the F_7 generation (6·2 days) with a temperature of 16–17° C. is lower than either the F_3 or F_5 generations when the temperatures were higher (17–20° C.) and the average survival 7·5 and 10·5 days respectively; while other generations, F_9, F_{11}, at lower temperatures than the F_7, had longer average survival times. Similarly, there was great variation in the *maximum* length of life of the females of different generations. In F_7 the maximum was 8 days, while in F_5 and F_8 it was 19 days. Thus, out of 254 unfed females none lived longer than

19 days, and the average length of life was much less (Fig. 2). In no case was any "autotrophic" development of fat-body, as described by Roubaud, observed in the unfed females, and there was no evidence from these experiments that cyclical hibernation (asthenobiosis) occurs in this race under laboratory conditions independently of temperature and food.

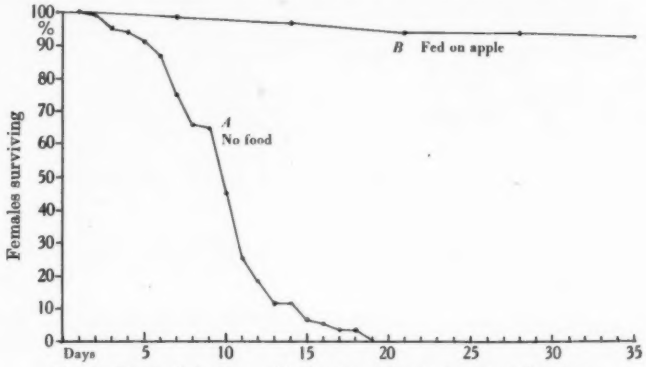

Fig. 2. Survival, in days, of fed and unfed anautogenous females.

On the other hand, females kept under similar conditions except that they were fed on fruit, rapidly accumulated fat-body and their lives were greatly prolonged. The difference between the survival rates of the fed and unfed females is clearly shown in the curves in Fig. 2, where curve *A* represents the survival rate of the unfed females and curve *B* the survival rate, for the first 5 weeks, of the females fed on fruit. Of the unfed females only 25 per cent.

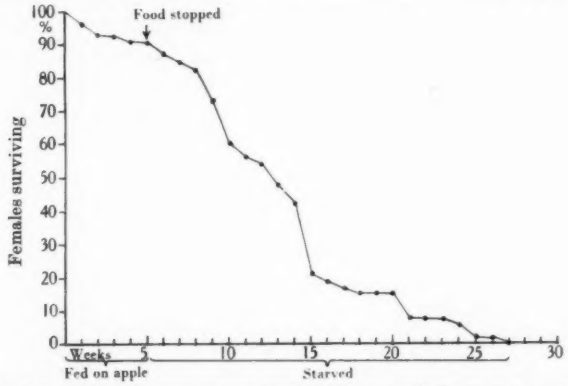

Fig. 3. Survival, in weeks, of anautogenous females fed on apple for 5 weeks and then starved.

survived until the eleventh day and none lived beyond the nineteenth day, but over 90 per cent. of the fed females lived for 5 weeks. The food was removed from the fed females after 5 weeks, when they had become very fat, and they were kept without food until they died (Fig. 3). Nevertheless, 50 per cent. of

them lived for 13 weeks, that is, 8 weeks of starvation, and 15 per cent. survived 15 weeks' starvation, or 20 weeks in all. The remaining 14 per cent. died off gradually until the last died 27 weeks after the experiment was started (Fig. 3). Thus, when females of this race are fed on apple, in 5 weeks they can store up sufficient reserves to allow about 15 per cent. of them to survive over 4 months of starvation, which is long enough to carry them through the severe winter months.

These results agree with the view of Roubaud (1933) and Wesenberg-Lund (1920–1) that hibernating females of *C. pipiens* do not require a blood meal for fat-body formation. Our results differ from Roubaud's as regards the formation of fat-body without food in the imaginal stage. In our experiments fat-body was not developed unless the females were fed on fruit for some weeks after emergence.

(5) *Absence of gonotrophic dissociation in hibernating females of the English anautogenous race*

When hibernating females were induced to gorge by prolonged illumination they oviposited readily after a single blood meal, and there was no evidence of "gonotrophic dissociation" such as Roubaud (1933) describes in post-hibernating females. In experiments done in December sixty-two females gorged after illumination, and fifty-three (=85 per cent.) of them laid egg rafts. The small proportion of females which gorged without illumination also laid normally after a single blood meal. Thus, in six experiments done in January, February, April and May, seventy-three females gorged and fifty-seven (=78 per cent.) of them laid rafts. It is remarkable that while 78 per cent. of the hibernating females laid after a single blood meal, of 256 active females which gorged only 149 (=58 per cent.) laid egg rafts. Hence, in the English anautogenous race of *C. pipiens* gonotrophic dissociation, if it occurs at all, can do so in only a small percentage of the hibernating females, as about 80 per cent. of them lay normally after only one blood meal.

(6) *Gorging in relation to pairing*

Woodcock (1914) considers that normally one blood meal is taken before and one after pairing. Weyer (1934*a*) says that usually pairing is necessary before gorging takes place. In our experiments the fact that a large percentage —up to 67 per cent.—of the rafts laid are infertile strongly suggests that unpaired females will gorge readily, because almost certainly the infertile rafts originated from females which had not paired. The view that gorging is independent of insemination is definitely proved by the results of the light and dark experiments Nos. 4, 9, 10 and 11, as unpaired females were used in these experiments, and, under favourable conditions, 56–85 per cent. of them gorged. It is probable that under natural conditions pairing occurs soon after the emergence of the adults, and that the females are usually inseminated before they take blood meals. This is certainly the case with hibernating females which are

always inseminated when they are collected during the winter, although they do not normally take blood meals until the following spring.

It is probable that insemination favours maturation of the ova and stimulates oviposition. This is suggested by the fact previously mentioned that when rafts are laid by a large batch of gorged females most of the fertile rafts are among those laid soonest and few of the rafts laid later hatch. The stimulating effect of insemination may also be the explanation of the difference between the average period required for maturation of ova in the autogenous and anautogenous races. This point will be dealt with later.

(7) *Pairing habits of the English anautogenous race*

The large cage necessary to obtain fertile eggs in this race is due to the mating habits of the adults. Towards evening, or in dim light, the males swarm, or dance in mid-air, and when females enter the swarm they are pursued by males and pairing begins in the air, although frequently the pair fall to the ground where copulation is completed. Males of this strain do not attempt to pair with resting females, even if they alight near or upon them, and they never attempt to pair with females when confined in small spaces. In fact, this race is "eurygamous" according to the terminology of Roubaud.

(8) *Absence of autogeny in the eurygamous English race*

In each of the eleven generations throughout the time of continuous breeding, a number of females was placed in small cages standing over dishes of water and kept in a thermostat at 24° C. until they died to test for "autogeny". In no case were any rafts of eggs laid by these females, and the absence of autogeny is also shown by the fact that in the large breeding cage egg rafts were never laid until after canaries had been put in for the females to gorge upon. In a previous experiment another strain of English *C. pipiens* was bred in the laboratory continuously for a year, and during that time there was no evidence of autogeny. Several other English strains were bred for shorter periods with similar results. It is, therefore, evident that this race breeds true through at least eleven generations.

(9) *Larval food and autogeny*

Recently de Boissezon (1933, 1934) has claimed that autogeny in *C. pipiens* is not a racial characteristic but is dependent on the larval food. This author considers that if the larvae are fed on food rich in proteins and iron the resulting females are autogenous; and he states that cooked lentils constitute such a food. This view is contrary to that of most other workers, and it has been disproved by Roubaud (1934) and Roubaud and Mezger (1934). We have tested de Boissezon's lentil medium by feeding a number of batches of larvae of our anautogenous race on it, but, although the larvae grew well and produced vigorous adults, in no case did any of the resulting females lay eggs without blood meals. The view that autogeny is not a racial characteristic but is

dependent on larval food is also disproved by our experiments in which anautogenous and autogenous races have been kept for many generations under similar conditions and fed on the same food (boiled yeast, brown bread, or dog biscuit) and have retained their distinctive characters throughout. We may mention that lentils form a very good food for larvae, but, in our experience, cooking is unnecessary and they are equally good, and more convenient for use, if the dried lentils are ground into a fine powder, a little of which is sprinkled on the surface of the water containing the larvae every day or two.

(10) *Biting habits of the English anautogenous race*

The females of the anautogenous race will bite man but they do so much less readily than they bite birds. Even when cages contain numerous females which attack birds voraciously, manipulations can be carried out within the cages with little risk of the hands being bitten. This is in striking contrast to the females of the autogenous race which attack man voraciously, and it is difficult to put a hand into a cage populated with them for even a brief interval without its being attacked.

III. Biology of the autogenous race of *Culex pipiens*

(1) *Origin and maintenance in the laboratory of three European strains of autogenous* Culex pipiens

During the past three years we have maintained in the laboratory three strains of autogenous *C. pipiens* originating from Greece, Malta and Hungary. These strains will be referred to as strains G, Greek; T, Maltese; and H, Hungarian, respectively. In a previous paper (1934) we described the manner in which we obtained these strains, and it is unnecessary to repeat it here. The important point is that none of them originated in a large town, and they all came from rural or semi-rural areas. Marshall and Staley (1935) have recently described an autogenous strain from the south of England which was not found in a large town. It is clear, therefore, that the autogenous race of *C. pipiens* is not confined to urban areas as Roubaud (1933) considers to be the case in France, but that the autogenous race has a wide geographical range and may be found in rural districts, as well as in towns, if the local conditions are suitable. It is probable that the autogenous race occurs commonly in rural areas in the south of Europe, and that farther north it finds in cities suitable conditions, in limited habitats such as cesspit, drains in cellars and cisterns in centrally heated buildings, where it can live and multiply throughout the winter. As yet it is not known how, or where, it passes the winter in those places, England and Hungary, where it has been found in the open in the summer and where the winters are too severe to allow continuous breeding in the open throughout the year.

The technique for breeding the larvae was the same as that already described for the anautogenous race. The adults, however, were not kept in large cages but in small cages formed of gauze-covered cylinders, 15 cm. high,

surmounting dishes, similar to those used for raising the larvae, containing water. Both the larvae and adults were kept in a thermostat at 24° C. Under these conditions eggs of both races hatch in about 48 hours, the length of larval life is about 12 days, although the first pupae are usually formed on the tenth day and some larvae may remain in the fourth stage long after the majority have pupated; the length of pupal life is about 48 hours; and females begin to lay about 4 days after emergence. Thus the complete cycle, from egg to egg, takes about 3 weeks. 100–200 larvae, or more, may be reared in a single dish if the water is changed daily and sufficient food is supplied. It was found constantly that the pupae which are formed on the first day or two mostly give rise to male adults, and that the pupae formed later give rise mostly to females. When the ratio of males to females which emerged was compared for a large number of different cultures of larvae, through several generations, it was found that on the average about equal numbers of both sexes emerge, although occasionally cultures may produce many more of one sex than the other.

By these methods the three strains have been maintained breeding continuously in the laboratory for three years. During this time strain G has passed through forty-nine generations, strain H through forty-four generations, and strain T through forty-seven generations, in all cases without any blood meals. Although different batches may show great variation in size and vigour according to the exact conditions under which they are raised, such as the degree of crowding and the abundance of food, there has been no obvious progressive decrease in the stamina of the strains which are still breeding vigorously.

The breeding stock of the strains have had no blood meals throughout all these generations, although sometimes the adults were fed on apple when it was necessary to keep them alive for experimental purposes. However, as our experience confirms MacGregor's (1932) view that food in the adult stage, other than blood, does not influence the laying of autogenous rafts, we may consider that our strains have been maintained "autogenously" for the number of generations given above. These results prove definitely that blood meals are not essential for the breeding of autogenous *C. pipiens*, and that they can breed continuously for at least forty-nine generations without recourse to blood.

(2) *Spanogyny*

Roubaud (1933) considers that when cultures are kept for a long time without blood meals they tend to die out, and this is manifested by a gradual and progressive decrease in the number of females produced in succeeding generations. To this phenomenon Roubaud gave the name *spanogyny*. Our experiments show that while spanogyny may occur if the conditions of growth are not favourable, it does not necessarily occur over a period of 3 years comprising forty-nine generations if the cultures are kept under very favour-

able conditions. We had one case in which it occurred in strain H when it was kept for some time at room temperature and the number of individuals became low. In the thirty-seventh generation 100 males and only one female emerged. This female laid a very small egg raft from which one male and two females were obtained (thirty-eighth generation). Two rafts were laid by these females and the resulting larvae, which were kept under good conditions as regards food and space, developed into twenty-five males and twenty-seven females (thirty-ninth generation). Since then the strain has progressed normally and it is now in the forty-seventh generation and shows no preponderance of males or other evidence of loss of stamina. This shows clearly that spanogyny, if it does arise, is not always due to a progressive decrease in vitality but may be a temporary reaction to unfavourable conditions which ceases in succeeding generations if the conditions of growth are improved. It is certainly not an invariable result of prolonged breeding without blood meals in the adult state resulting in depletion of nitrogenous reserves and nitrogen starvation, as has been described by Roubaud in his strains (1933).

(3) *Pairing and oviposition in the autogenous race*

It has been shown that in the anautogenous race oviposition is not dependent on previous insemination. This also applies to the autogenous races, and eggs may be laid by unpaired females as was noted by MacGregor (1932). We confirmed this by isolating pupae in test-tubes in which the resulting imagines were kept until they died. In one experiment nine pupae of strain G were isolated and produced eight females, four of which laid small rafts autogenously and without any possibility of their having paired. It was also found that such isolated and unpaired females of the autogenous race would gorge so that, as in the anautogenous race, pairing and gorging are mutually independent.

(4) *The autogenous egg rafts*

We agree with previous workers that the egg rafts laid autogenously are small and triangular in shape. They rarely contain more than 100 eggs and, although according to the conditions of growth of the larvae as regards food and crowding, the number of eggs per raft may vary from twenty to more than 100, the usual number per raft is fifty to eighty. If, after depositing the autogenous rafts, the females are given blood meals they then lay a second time, these rafts being larger than the autogenous ones and containing about twice as many eggs (the average number per raft according to MacGregor (1932) is 150). Thus, if the numbers of eggs in the autogenous and blood rafts are added together it appears that each female lays, with one blood meal, a total of about 200–250 eggs. This is about equal to the number of eggs laid by a female of the English anautogenous race after one blood meal, although sometimes such females may lay very large rafts containing 300 or even 400 eggs.

Experiments extending over many generations have shown that, while the

food of the larvae may control the proportion of females which lay autogenous rafts and the size of the rafts, autogenous oviposition cannot be induced in an anautogenous race by varying the larval food. As regards racial differences, however, the crucial point is that when the larvae of the two races, autogenous and anautogenous, are reared under similar conditions and given the same food, one race regularly produces females which lay without any food in the adult stage, but the other race does not, even in the course of many generations, produce females which lay without food in the adult stage, and they must always have food in the form of blood before oviposition.

In such work it is important that conclusions are drawn only from large numbers of individuals and from series of experiments extending over several generations, as we have found that the proportion of females which lays

Table IV. *Percentage of females of strains G and T laying autogenously*

Strain and generation	No. of females	No. autogenous rafts laid	Percentage of females which laid
G, F_2	22	12	54·5
G, F_3	43	28	65·1
G, F_4	151	116	76·8
G, F_5	83	68	81·9
G, F_6	128	110	85·9
G, F_6	233	148	63·5
G, F_7	132	57	43·1
G, F_8	259	106	40·9
Total	1051	645	61·3
T, F_3	156	121	77·5
T, F_4	122	95	77·8
T, F_5	231	104	43·7
T, F_6	255	122	47·8
T, F_7	130	89	68·4
Total	894	531	59·4

autogenously may vary greatly in different generations, and even in different batches of the same generation. Careful records were kept of the number of females which emerged, and the number of autogenous rafts laid for a number of batches of larvae over four generations of strains G and T. These figures are summarised in Table IV. In no case where large numbers were dealt with did all the females lay egg rafts. In strain G the percentage of females which laid varied from 40·9 to 85·9, and of a total of 1051 females 645 laid rafts giving an average of 61·3 per cent. For strain T the percentage of ovipositing females varied from 43·7 to 77·8 per cent. and the average for 894 females is 59·4 per cent.

(5) *Oviposition by autogenous females after a blood meal*

In spite of being maintained for many generations without blood meals the autogenous females do not lose the ability to oviposit after a blood meal. In Table V are given the number of females which gorged and the number of egg rafts laid for a number of generations of strains G and T. For strain G figures are given for eight generations beginning at the sixth and extending at intervals

until the forty-second. In all, 339 gorged females laid 255 rafts, which is equal to 75 per cent. of the females ovipositing. In strain T figures for eleven generations, at intervals from the fourth to the thirty-eighth, are given, and, of a total of 507 gorged females, 369 (=73 per cent.) oviposited. These figures, for both strains, are plotted in Fig. 4 as percentage of gorged females ovipositing in successive generations, and from the curves it is seen that the percentage oviposition in response to a blood meal does not show a regular variation with the increase in the number of generations during which the strain has been maintained autogenously. There is considerable variation from generation to generation, but it is an irregular one and, in general, prolonged autogeny does not appear to have any definite adverse effect on the capacity of the females to lay after a blood meal.

Table V. *Percentage of females of strains G and T ovipositing after a single meal of blood*

Strain and generation	No. of females gorged	No. of rafts laid	Percentage of gorged females which laid
G, F_6	46	43	94
G, F_9	11	10	91
G, F_{11}	86	67	78
G, F_{17}	23	16	70
G, F_{25}	41	25	61
G, F_{31}	58	37	64
G, F_{39}	43	31	72
G, F_{42}	31	26	84
Total	339	255	75
T, F_4	33	28	85
T, F_6	81	48	59
T, F_7	107	94	88
T, F_9	21	16	76
T, F_{11}	60	45	75
T, F_{14}	36	28	78
T, F_{15}	26	12	46
T, F_{23}	71	52	73
T, F_{25}	24	15	63
T, F_{26}	26	19	73
T, F_{38}	22	12	55
Total	507	369	73

The time taken by the ovipositing females to mature ova after a blood meal is plotted in Fig. 5 for strains G and T of the autogenous, and for the anautogenous English race. The percentages in the case of strain G are based on 265 ovipositions, in strain T on 379 ovipositions, and in the anautogenous race on 248 ovipositions. It is evident that maturation and oviposition take place rapidly and regularly in the autogenous strains. Oviposition begins on the third day, one raft only was obtained from a female of strain T on the second day, and by the seventh day 98 per cent. of the rafts have been laid. A few rafts appear later, but in these experiments all the rafts had been laid by strains G and T by the twelfth day. There is a great contrast in the behaviour of the anautogenous females which are very irregular in the interval between gorging and oviposition. Only 50 per cent. of them laid by the seventh day and even by the twelfth day 17 per cent. of the rafts had not been laid. These

17 per cent. of rafts appeared at very long and irregular intervals in a sporadic manner, so that some rafts were laid more than 28 days after the blood meal.

In comparing the oviposition of the autogenous and anautogenous races, however, the possible effect of pairing must be taken into account. In such

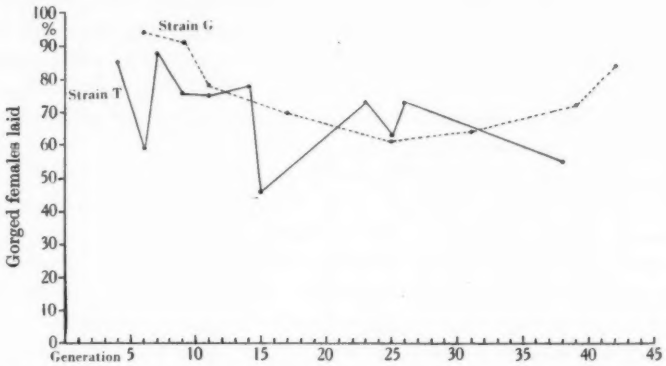

Fig. 4. Percentage of autogenous females laying rafts after a blood meal after an increasing number of generations of autogenous development.

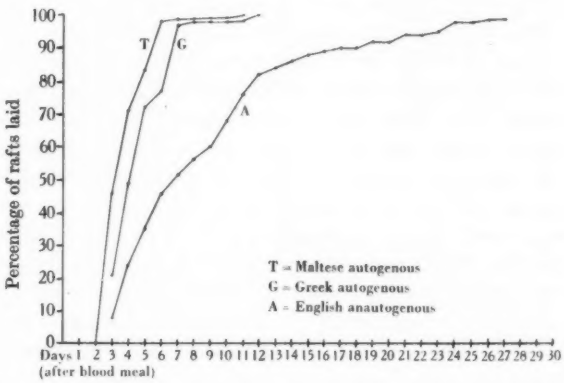

Fig. 5. Time taken for maturation of ova after a blood meal by females of the Greek and Maltese autogenous races and of the English anautogenous race.

experiments nearly all of the autogenous females are inseminated, while only a small proportion, if any, of the anautogenous females are inseminated. It is probable, as has been mentioned before, that paired females lay more readily than unpaired ones, and the irregular maturation times of the anautogenous females may be due to their not having paired.

(6) *Biting habits of the autogenous race*

Females of the autogenous race are, under suitable conditions, voracious biters of both birds and man. They gorge most readily about 7–10 days after emergence and usually appear reluctant to gorge before they have laid the autogenous rafts. Preferably they bite birds between the scales on the toes and legs, but they may attack around the eyes and anus also, or elsewhere if the feathers are removed. In this respect they resemble anautogenous females which also bite the toes and legs of birds in preference to other parts. The autogenous females, as has been described by Roubaud (1933), attack man very voraciously in contrast to the anautogenous females which show very little inclination to gorge upon man.

There is great variation between different lots of autogenous females, as regards their readiness to gorge, even when they have been reared under apparently similar conditions, and the biting experiments are done as nearly as possible under comparable conditions. The best results were obtained when the females were kept in a thermostat at 24° C. and the biting experiments were done at 20–24° C. As long as the atmosphere is humid, 70–80 per cent. relative humidity, the relative humidity does not appear to be a limiting factor, and a saturated atmosphere is not necessary for a high percentage of gorging. The females were usually kept in the dark but would gorge at any time during the day, in darkness or dim light, if given an opportunity. For convenience most of the biting experiments were done at night.

The bloodthirstiness of the autogenous females of strains G and T towards birds was tested at intervals through forty-three generations for strain G (females from thirteen generations tested) and through forty generations for strain T (females from seventeen generations tested). The results are given in Table VI, and it is seen that of the 763 females of strain G tested 442, or an average of 58 per cent., gorged at the first opportunity. For strain T the corresponding figures are: 1016 females tested, of which 557, or an average of 55 per cent., gorged. The great variation in the percentage of females which gorge in different generations is noticeable, but, except for a possible slight decrease in bloodthirstiness of strain T in the later generations, the long periods and numerous generations during which the strain was kept breeding autogenously do not appear to have influenced significantly the biting habits of the females. This is best seen if the totals for the generations up to the twentieth and of those after the twentieth are compared. Of strain G, for the ninth to the twentieth generations, 184 out of 350 females gorged, giving an average of 53 per cent.; and, for the twenty-fifth to forty-third generations, 258 out of 413 females gorged, giving an average of 63 per cent. With strain T, for the generations fourth to sixteenth, 384 out of 638 females gorged, an average of 60 per cent.; while for generations twenty-third to fortieth, 173 out of 378 gorged, an average of 46 per cent. Thus in the case of strain G there appears to have been a small increase in bloodthirstiness with prolonged autogeny; but

in strain T there appears to have been a decrease in bloodthirstiness. However, the variations from the mean averages for the two strains of 58 and 55 per cent. respectively is so small that the figures cannot be regarded as proving a definite change in either direction.

Table VI. *Effect of prolonged autogeny on the biting habits of females of the autogenous race of* Culex pipiens

Strain	Generation	No. females	No. gorged	% gorged
G	F_9	44	11	25
G	F_{11}	104	86	83
G	F_{14}	58	9	16
G	F_{15}	50	17	34
G	F_{17}	48	23	48
G	F_{20}	46	38	83
	F_9–F_{20}	350	184	53
G	F_{25}	44	41	93
G	F_{25}	43	10	23
G	F_{27}	54	44	82
G	F_{31}	66	58	88
G	F_{34}	24	11	46
G	F_{39}	64	43	67
G	F_{42}	61	31	51
G	F_{43}	57	20	35
	F_{25}–F_{43}	413	258	63
	F_9–F_{43}	763	442	58
T	F_4	52	33	64
T	F_6	117	81	69
T	F_7	122	107	88
T	F_9	46	21	46
T	F_{11}	68	60	88
T	F_{13}	65	9	14
T	F_{14}	51	36	71
T	F_{15}	57	26	46
T	F_{16}	60	11	18
	F_4–F_{16}	638	384	60
T	F_{23}	106	71	67
T	F_{25}	60	24	40
T	F_{26}	46	26	43
T	F_{37}	55	17	31
T	F_{38}	64	22	34
T	F_{40}	47	13	28
	F_{23}–F_{40}	378	173	46
	F_4–F_{40}	1016	557	55

As has been mentioned previously, preliminary exposure to light does not significantly influence the gorging of the autogenous races. When during these experiments autogenous females were kept illuminated for several days, it was noted that they frequently began to oviposit immediately they were placed in darkness whatever the time of the day. This was found to be a convenient method of obtaining eggs of a known age. Jobling (1935) has recently studied the relation of light and shade to oviposition and finds that the females lay for preference on shaded water, and in darkness show preference for the nature of the medium, hay infusion being especially attractive. In our experiments the females were offered either tap water or wet absorbent paper for oviposition.

They laid readily on the wet paper so that a free water surface is not essential for oviposition by *C. pipiens*.

Incidental observations during this work support MacGregor's (1931) view as to the function of the oesophageal diverticula. Normally blood passes directly into the mid-gut and water or fruit juices pass into the diverticula. Occasionally, however, probably owing to back pressure, blood is found in the diverticula, and in many instances the diverticula of newly gorged mosquitoes were found to be filled with blood of which the corpuscles were normal in appearance and were not plasmolysed. In this point we disagree with De Boissezon (1930*a*), who considers that the openings into the diverticula are too small to permit corpuscles to enter and says that only plasmolysed blood is found in the diverticula.

(7) *Pairing habits of the autogenous race*

The autogenous race differs greatly from the anautogenous race in its pairing habits. The adults of the anautogenous race always begin copulation on the wing, but in the autogenous race the males settle on resting females, force their way beneath them and copulate whilst the females remain at rest. It is very noticeable in cultures of autogenous *C. pipiens* that the slightest disturbance which causes the adults to fly induces pairing. For instance, if a cage of recently emerged adults is brought into the light from darkness, pairing begins actively and females may be surrounded by five or six males trying to pair. The same result arises from tapping the cage or blowing into it. Pairing may take place in a very small area, and in one experiment two generations of strain G were raised in a boiling tube about 2·5 cm. wide by 15 cm. long.

One male can inseminate more than one female, and from some experiments it appears that a single male is able to pair successively with several females, although all the eggs of the resulting rafts may not be fertile. In one case, with strain G, a single male was in a cage with fifty-six females. From them seven rafts were obtained of which some eggs of three rafts hatched, thus proving that the male had paired with at least three females.

(8) *Cross-mating of autogenous and anautogenous races*

Experiments have been done on cross-mating between the English anautogenous race and the Greek and Hungarian strains of the autogenous race. The results of the anautogenous + Hungarian cross have been published in part (Vincent, 1933), and it is only necessary to mention here that when H males were crossed with anautogenous females the progeny (F_1) were stenogamous, but not autogenous; and to add that, after publication of the results in 1933, autogeny appeared in the F_2 progeny.

Essentially similar results were obtained with the Greek autogenous + anautogenous cross. Greek males readily paired with anautogenous females in small cylinders, and the results of one cross-mating experiment which was

followed for ten generations are given in Table VII. Thirty Greek males (autogenous) were put in a small cylinder with twelve anautogenous females (English). Pairing on the sides and floor of the cylinder was observed and, after gorging, two of the females laid fertile rafts. From these rafts eighty males and 103 females (F_1) were raised and the latter laid two small autogenous egg rafts which formed the basis of a cross-bred autogenous strain that was maintained in the laboratory for ten generations. The details of the further generations are given in Table VII, and it is seen that for the first three generations the percentage of females laying autogenous rafts was very small, 1·9–6·6 per cent., but it increased suddenly to 68·4 per cent. in the fourth generation, and in subsequent generations it fluctuated between 54·3 and 67·1 per cent., except in the fifth generation in which it fell to 17·2 per cent. Throughout

Table VII. *Cross-mating of Greek autogenous males with English anautogenous females*

Generation of progeny	No. of males	No. females	No. autogenous rafts	% of females laying	Rafts fertile no. = %
F_1	80	103	2	1·9	2
F_2	51	64	2	3·1	2
F_3	32	30	2	6·6	2
F_4	22	38	26	68·4	—
F_5	173	215	37	17·2	—
F_6	244	241	119	55·6	113 = 94·9
F_7	79	67	45	67·1	45 = 95·7
F_8	89	92	50	54·3	45 = 90·0
F_9	111	87	56	64·3	46 = 82·1
F_{10}	107	81	44	54·3	40 = 90·9
F_1–F_{10}		1018	383	37·6	295 = 92·0

the experiment nearly all the autogenous rafts hatched (an average of 92 per cent.), which shows that the cross-bred adults were stenogamous. In the F_1 generation thirty-six females gorged and twenty-four of the resulting thirty-one rafts were fertile; while in the F_3 generation twenty-seven out of thirty-six rafts laid by gorged females were fertile. Thus it appears that in the cross-bred adults stenogamy appears in most of the progeny, if not all, even in the F_1 generation, whilst autogeny is exhibited by only a very small proportion of the progeny of the first three generations. This cross-bred strain was also maintained for four generations by rafts laid by gorged females, and autogenous rafts were laid by some of the females of the F_2 generation (one raft from eighty-five females) and of the F_3 generation (eleven rafts from 253 females).

Cross-mating in this direction, autogenous males + anautogenous females, was readily obtained in several other experiments.

The reverse cross, anautogenous males + autogenous females, was also obtained, but only in large cages in which the males had room to swarm in mid-air. In one experiment 696 English anautogenous males were put in a large cage with eighty-two autogenous females (strain G) which had been isolated as pupae in test-tubes to preclude any possibility of stray autogenous males being introduced into the large cage. From them fifty-one autogenous

rafts were obtained, all of which hatched, that is 100 per cent. fertility, although with the English anautogenous females under similar conditions we never obtained more than about 50 per cent. of fertile rafts. In the F_1 generation, by 135 females, twelve autogenous rafts were laid. Only five females of the F_2 generation were raised, and one of them laid an autogenous raft. In the F_3 generation fifty females were raised and seven autogenous rafts were laid by them, all of which hatched. This strain was then abandoned. Thus the result of crossing anautogenous males with autogenous females appears to be very similar to that described above for the reverse cross. The progeny from the first generation are preponderately stenogamous, but autogeny is shown by only a small percentage of individuals in the early generations and increases in an irregular manner later.

These results differ greatly from those obtained by Roubaud (1933) with French autogenous and anautogenous races. Roubaud and his co-workers found it difficult, or impossible, to cross-mate autogenous males with anautogenous females, although they could easily obtain the reverse cross, anautogenous males with autogenous females. They found that the progeny of the latter cross lost the capacity for autogeny in the first generation but regained it to a small extent in the second generation, and they, hence, concluded that autogeny is a Mendelian recessive character. Roubaud (1933) has, however, described another race of *C. pipiens* from Algeria which is stenogamous but anautogenous, and the females of which are readily fertilised by autogenous males.

It is evident, therefore, that autogeny and stenogamy behave as definite genetical characters when the different races are cross-mated; and that the problem of races in *C. pipiens* should lead to very interesting results if investigated on genetical lines by carefully controlled cross-mating experiments.

(9) *Effect of low temperatures on eggs, larvae and adults*

To test the possibility of keeping eggs unhatched for long periods experiments were made on the survival of eggs at low temperatures. The results were, however, irregular and inconclusive. The results of the experiments on eggs, together with some on the survival of larvae and adults, are given in Table VIII. The experiments were done in cold chambers constant to within 0·5° C., except for the first which was done in a small refrigerator. The most interesting point in the experiments is that the first lot of eggs, English anautogenous, not only survived for 9 days at about + 2° C., but hatched at that temperature and, when the larvae were transferred to room temperature, they grew well and developed into normal adults (Exp. 1). On the other hand, with the Hungarian autogenous strain only one out of two rafts hatched after 2 days at + 5°C., and after 4 days only one larva hatched from two rafts. After 5, 6 and 7 days no hatching took place (Exp. 2). At + 1° C., out of twenty-four rafts, two were removed after 1 day and did not hatch, two failed to hatch after

2 days, and the remainder were dead when tested after 3 days (Exp. 3). (In these experiments the eggs were tested for viability by being removed from the low temperature, gradually allowed to come to room temperature, and then placed in a thermostat at 24° C.) In Exp. 4 about thirty rafts were placed at 0° C., and two of them were removed and tested for viability after from 1 to 8 days but none hatched. The experiment was repeated, No. 5, using two lots of eggs, one (*a*) less than 24 hours old, and the second (*b*) more than 24 hours old. After 24 hours at 0° C., two out of thirty-five of the older (*b*) rafts hatched, but

Table VIII. *Effect of low temperature on eggs, larvae and adults of* Culex pipiens

Exp.	Material	Temp. °C.	Period at low temperature days	Result
1	Eggs, anautogenous	+2	9	Hatched 9th day
2	Eggs, autogenous Strain H	+5	1	1/2 rafts hatched
			4	1 larva from 2 rafts
3	,,	+1	1, 2, 3	0/24 rafts hatched
4	,,	0	1–8	0/30 ,,
5	Eggs (*a*) less than 24 hours old	0	1	0/35 ,,
	(*b*) more than 24 hours old	0	1	2/35 ,,
6	Eggs, autogenous Strain H	−5	2	1/2 ,,
			3–7	0/28 ,,
7	,,	−10	1–3	0/10 ,,
8	First stage larvae, strain H	0	1	All dead
9	Hibernating adults, English	−16	4	All dead (11)
10	Adults, bred in laboratory, English	−16	4	All dead (11)

all the young (*a*) rafts were dead. This experiment indicates that eggs are more resistant when they are nearly ready for hatching than when they are newly laid. In Exp. 6, thirty rafts were put at −5° C., and one out of two of them hatched after 24 hours but none hatched after 72 hours or longer. In Exp. 7, none of ten rafts survived 24 hours or longer at −10° C.

In Exp. 8, first stage larvae of strain H were put at 0° C., but they were all dead within 24 hours. Two experiments were done to test if adults could survive a very low temperature. In Exp. 9, eleven hibernating English females were put at −16° C., but they were all dead within 4 days. Similarly in Exp. 10, eleven females bred in the laboratory of the English anautogenous strain failed to survive 4 days at −16° C.

These few preliminary experiments indicate that while ripe eggs may survive for a short time temperatures down to −5° C., there is no possibility of their surviving throughout the winters of northern or central Europe in exposed water. Young larvae also appear to be very susceptible to low temperatures, but it is possible that fourth-stage larvae may be more resistant. Even hibernating adults of the anautogenous race are quickly killed by very low temperatures (−16° C.), so that to pass severe winters they must seek sheltered resting places where the temperature fluctuations are less than in the open. It is difficult to understand how the autogenous race passes the winter in rural

areas where habitats such as cesspits which would permit of continuous breeding are not available. Under laboratory conditions the autogenous females do not accumulate large reserves of fat-body and usually die within a month or two, even when fed on fruit. It is possible that survival is dependent on very slow breeding of a few individuals in very restricted and sheltered areas, such as in drains in animal houses.

IV. Discussion

The general result of our work is to confirm the conclusions of Roubaud and his co-workers that there are two distinct races of *C. pipiens* in Europe. The one race, *C. pipiens pipiens* of Roubaud, is a hibernating, eurygamous race, the females of which require a blood meal before oviposition (anautogenous); while the second race, *C. pipiens autogenicus*, of Roubaud, is non-hibernating and stenogamous, and the females lay fertile eggs without any food in the adult stage, that is, they are autogenous. We consider, however, that the application of the term "autogenous" to this race has been unfortunate, as it has led some other workers to regard "autogeny" as the only character differentiating the two races, and to neglect the other important differences. As our results show, autogeny is a variable character, inasmuch as on the average only about 60 per cent. of females lay autogenous rafts, and under unfavourable conditions only very few may do so. We consider that eurygamy and stenogamy are more constant characters for differentiating between the two races. Recently additional differences of a morphological nature have been described in the egg floats of the two races by Roubaud (1935) and in the chaetotaxy of the larvae by Marshall and Staley (1935).

In comparing the races it is essential that all the characters should be taken into consideration, and that conclusions should be drawn only after the strains dealt with have been bred for several generations under conditions such as preclude the possibility of interbreeding. Another point which has led to confusion is the fact that Roubaud described the autogenous race as an *urban* one and this has been understood by some authors to imply that it is never found in rural areas and that, consequently, *C. pipiens* collected in rural areas are always of the anautogenous, eurygamous, race. In fact, we have described autogenous races from rural areas in Greece, Malta and Hungary, and recently Marshall and Staley (1935) have found an autogenous race in a rural part of the south of England. Thus, although owing to the local conditions being favourable the autogenous race may breed freely within towns where conditions are not suitable for the anautogenous race, it is not confined to such habitats but occurs also in rural districts. It is possible, and is strongly indicated by Vincent's (1933) results with a Hungarian strain of *C. pipiens*, that both the autogenous and the anautogenous races may occur together in the same locality in rural districts. The results of cross-mating experiments show that interbreeding is possible and it may take place in nature where the two races coexist. Probably De Boissezon's results, which are so contradictory to those of

other workers, are due to the presence of the two races in rural districts in the south of France where his work was done.

Our results differ from those of Roubaud in that we found that the English anautogenous race, unlike the French one, is not subject to obligatory, cyclical, hibernation and cannot store up fat-body at the expense of larval tissues without food in the adult stage. We agree with Roubaud (1933) and Wesenberg-Lund (1920–1) that blood meals are not necessary for fat-body development, but we have found that carbohydrate food in the form of fruit juice is essential for the development of fat-body in adults of the English anautogenous race[1]. Our experiments have also shown that light is an important factor in the life history of the hibernating race and that it exerts a very definite influence on the activity of the females. With sufficient illumination they remain active and gorge freely, but with decreased illumination they tend to accumulate fat-body and refuse to gorge. These results suggest that under natural conditions light may influence hibernation and that, perhaps, in the anautogenous race the length of daylight may be an important factor in inducing hibernation in the autumn generation.

In any case, by employing additional illumination during the winter, we have succeeded in breeding the anautogenous race continuously, uninterrupted by cyclical hibernation, although under natural conditions hibernation undoubtedly takes place. De Buck and Swellengrebel (1934) have recently shown that *Anopheles maculipennis messeae* can be kept breeding continuously in the laboratory although it hibernates under natural conditions. This is very similar to what we found with the anautogenous race of *Culex pipiens* except that in this case, in addition to the temperature factor, the period of illumination is of primary importance, and without additional light continuous breeding is not possible in the winter months.

The main points which emerge from our work on the autogenous race is that prolonged autogenous development over a period of 3 years does not entail loss of vigour in spite of strains passing through forty-five to forty-nine generations during this period. In this we differ from Roubaud (1933), who considers that prolonged autogenous development results in nitrogen depletion and, unless the females are allowed to gorge, to a gradual and progressive decrease in vigour manifesting itself by the appearance of "spanogyny" in the cultures, that is, by a progressive decrease in the number of females produced and hence a gradual diminution of the breeding stocks. Our experiments prove definitely that, if the breeding stocks are kept under good conditions, blood meals are not essential for the maintenance of stamina in the autogenous race, and breeding can continue for at least fifty generations without deterioration of the stocks.

The results of our experiments on the cross-mating of autogenous and anautogenous races also show interesting differences from those recorded by

[1] Since this paper went to press, De Buck (1935) has published results for Dutch *C. pipiens* which agree with our results for the English anautogenous race.

Roubaud (1933). We readily obtained crosses in both directions, that is to say, male autogenous + female anautogenous and male anautogenous + female autogenous. Roubaud, on the other hand, found it difficult, or impossible, to obtain the former cross, although he easily crossed anautogenous males with autogenous females. The cross-mating experiments show clearly that autogeny and stenogamy are genetical characters and are transmitted to the progeny. Stenogamy appears in the F_1 generation, but autogeny may not appear until the F_2 generation and then in only a small percentage of the females, although later, in the fourth or fifth generation, it may increase suddenly to over 60 per cent. The suggestion of Roubaud (1930*a*) that autogeny behaves as a Mendelian recessive character is not borne out by our experiments, since in one case (Table VII) autogeny appeared in the F_1 generation. Much further work on genetical lines is necessary, however, before the mode of inheritance of the racial characters of the two races can be discussed with confidence.

V. Summary

1. An English anautogenous race and three European strains of the autogenous race of *Culex pipiens* have been studied comparatively for 3 years under laboratory conditions.

2. Prolonged illumination has an activating influence on females of the anautogenous race, both hibernating and laboratory bred, and greatly stimulates gorging.

3. With the aid of artificial light during the winter months the anautogenous race has been kept breeding in the laboratory throughout the year for eleven generations without the occurrence of cyclical hibernation (asthenobiosis). Consequently it is suggested that the length of daylight may be a factor of importance in controlling the natural hibernation of females of this race.

4. Females of the English anautogenous race, whether of hibernating, or of active generations, laid normally after one blood meal and did not exhibit gonotrophic dissociation. They also oviposited readily after being artificially fed on bird blood.

5. Unfed females lived for a maximum of 19 days and never accumulated fat-body autotrophically.

6. Females, if fed on apple for 5 weeks, accumulated sufficient reserves to enable a small percentage of them to survive 15 weeks of starvation.

7. In the anautogenous race pairing always begins in the air although it may be completed on the ground. The females do not lay without a blood meal, and they show little tendency to bite man but bite birds voraciously.

8. Strains of the autogenous race of *C. pipiens* were obtained from rural areas in Greece, Malta and Hungary and have been maintained in the laboratory for over 3 years.

9. Continuous breeding under autogenous conditions (no blood meals) for several years and through forty-five to forty-nine generations has had no deleterious effect on these autogenous strains.

10. Spanogyny, or the gradual decrease in the numbers of females produced in succeeding generations, does not necessarily follow prolonged autogeny if the cultures are kept under favourable conditions.

11. Males of the autogenous race pair with resting females, so that pairing can take place in very small areas.

12. Cross-mating was easily obtained between the two races, autogenous and anautogenous, in both directions: male autogenous + female anautogenous; and male anautogenous + female autogenous. Stenogamy and autogeny are hereditary characters. Stenogamy always appears in the F_1 generation, but autogeny sometimes appears in the F_1 generation and sometimes not until the F_2 generation.

13. Eggs are quickly killed by temperatures below freezing-point; and young larvae die within 24 hours at 0° C. Adults, even hibernating females, die within 4 days at −16° C.

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COCCIDIOSIS OF THE GREY AND STONE PARTRIDGE

By W. L. YAKIMOFF and I. L. MATIKASCHWILI

(*From the Parasitological Laboratory of the Veterinary School, Leningrad*)

Through the kindness of H. H. Woken, the veterinary surgeon of the Zoological Garden in Leningrad, we were able to examine the faeces of two species of birds—the grey partridge (*Perdix perdix*) and the stone partridge (*Caccabis chucar*). It is very probable that the coccidian found by us is common to both species of birds, but as they were kept in the same cage it was not possible to determine the origin of the faeces examined.

Darling's method was used for the concentration of the oocysts. These were oval in shape, but occasionally spherical, and the envelope had a double contour. A polar granule was seen but there was no residual body. The size of the oval forms (calculated from 48 oocysts measured) is given in Table I.

Table I

	Oocysts			Form index	
	Length	Breadth	Average		Authors
Perdix perdix	24·0–32·0	12·0–21·9	29·1 × 16·7	—	Verwey (1926)
Lagopus scoticus	25·0–35·0	14·0–20·0	—	—	Fantham (1910)
Lagopus lagopus	17·0–30·6	15·3–27·2	23·63–25·78 × 17·51–23·4	0·59–1·0	Brinkmann (1926)
Perdix perdix and *Caccabis chucar*	16·2–25·0	14·0–20·0	20·0 × 17·6	0·69–0·97	Yakimoff and Matikaschwili

We see that the size is 16·2–25·0 × 14·0–20·0 μ, with an average size of 20·0 × 17·6 μ. The maximum size is 25·0 × 20·0 μ and the minimum 16·2 × 15·0 μ. The most frequent size is 20·0 × 16·5 μ. The form index is 1 : 0·69–0·97 with an average of 1 : 0·84 and most frequently 1 : 0·82. The diameter of the spherical oocysts was 17·6 μ.

When the faeces were kept in a 2·5 per cent. solution of potassium bichromate four spores developed inside the oocysts. The spores were oval, pointed at one end, and measured 7·7–9·9 × 5·5–6·6 μ. Each sporocyst contained two sporozoites measuring 3·3–3·6 × 2·2–2·7 μ. Residual bodies were present in the sporocysts.

Coccidian parasites of nearly related species of birds have been described by Verwey (1926) in the partridge *Perdix perdix*, by Brinkmann (1926) in *Lagopus lagopus*, and by Fantham (1910) in the Scotch grouse, *Lagopus scoticus*. The size of the oocysts from these birds is given in Table I, and it is

seen that there is a difference between the coccidian found by us in *Perdix perdix* and *Caccabis chucar* and the other three forms. We consider that the coccidian found by us is a new species and we propose to name it *Eimeria kofoidi* n.sp. in honour of the American protozoologist, Prof. C. A. Kofoid.

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